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CONTENTS

Contribution to the study of <u>Streptomyces violascens</u> from the Australian forest environment (L. Gerrettson-Cornell).....	3
Studies on the powdery mildew fungus, <u>Sphaerotheca fuliginea</u> : Ultrastructure and host responses (Z. A. M. Baka, H. S. Aldesuquy)	15
El género <u>Alternaria</u> (Hyphomycetes, Deuteromycotina) en Cuba (A. Mercado Sierra, J. Mena Portales)	33
Nuevos o raros hifomicetes de Cuba VII. Especies enteroblásticas (A. Mercado Sierra, J. Mena Portales)	63
New names and new species in the Flora of Cuba and Antilles, IV (A. Borhidi)	75
A new <u>Erythroxylum</u> species in Cuba (R. Oviedo Prieto, A. Borhidi)	91
A review of Brazilian representatives of the <u>chrysotricha</u> species group in the genus <u>Tabebuia</u> Gomes ex DC. (Bignoniaceae) (J. Paclt)	95
<u>Ekmaniopappus</u> Borhidi gen. novum (Senecioneae, Asteraceae) in Hispaniola (A. Borhidi, E. Gondár, T. Kiss, Zs. Orosz-Kovács)	105
Phytoplankton studies in the Keszthely Bay (Lake Balaton, Hungary, 1983—1987) (É. Vízkelety)	119
Is there vegetation continuum in mangrove swamps? (Imoh Ukpong)	151
Ontogenetic characteristics in flowers of some plum cultivars (D. Surányi)	161
Xylotomical study of some Venezuelan tree species (Mimosaceae I—IV) (K. Babos, L. J. C. Cumana)	183
Architecture of the shoot system of <u>Quercus petraea</u> (Matt.)Liebl. (M. Nagy)	239
Cytological study of anthocyanin production in grapevine (<u>Vitis vinifera</u> L.) callus cultures (J. Jásik, B. Vančová)	251
Histomorphological studies of tracheary elements and chemical composition of <u>Khaya ivorensis</u> A. Juss. (Meliaceae) (E. E. Okoegwale, L. S. Gill)	261
Internal seed structure of selected vegetable species (R. Holubowicz, K. Ratajczak, K. Kryslak)	267
Preliminary studies on possible Ni-hyperaccumulator plants of Cuba (A. Borhidi, A. J. M. Baker, M. Fernandez Zequeira, R. Oviedo Prieto)	279
Simple in vitro propagation of insectivorous plants (M. E. Csányi, K. Benyő, E. K. Tóth)	287
Effects of carbon sources on the morphology and structure of <u>Scenedesmus acutus</u> Meyen (F. Nagy-Tóth, L. I. Péterfi, L. Fodorpataki)	295

Determination of radioactivities in some species of higher fungi (<u>E. Vaszari</u>)	317
Effects of phosphate application on carbohydrate partition and chloroplast ultrastructure of two <u>Capsicum annuum</u> L. varieties (<u>K. Margóczy, E. Takács, L. Técsi</u>)	325
Fungal spoilage of fresh pepper <u>Capsicum annuum</u> L. (<u>A. O. Ekundayo, B. A. Oso</u>)	337
Effect of the calmodulin-inhibitor, chlorpromazine on benzyladenine-induced shoot formation in derooted tobacco plants (<u>A. Ádám</u>)	343
Growth and alkaloid content characteristics of isolated roots of <u>Datura innoxia</u> Mill. (<u>N. Missaleva, G. Petri</u>)	351
Proline concentration, drought tolerance and water deficiency in wheat varieties (<u>S. Gulyás, G. Pálfi-Deim, L. Cseuz</u>)	359
Effects of copper on protein and biomass yields of rice plants (<u>F. C. Lidon, F. S. Henriques</u>)	373
Influence of laser beam of different wavelengths on the protein and nucleic acid content in germinating <u>Zea mays</u> L. (<u>I. Kerepesi, M. Tóth, L. Kozma</u>)	383
Dry matter accumulation and distribution, soil and ¹⁵ N labelled fertilizer nitrogen uptake and redistribution in maize (<u>S. Szlovák, K. Debreczeni</u>)	387
Interrelationship of <u>Oryza glaberrima</u> Steud. with annual wild rices of the <u>O. glaberrima</u> and <u>O. sativa</u> complexes: Analyses of variation in reproductive morphology (<u>F. M. Dania Ogbe</u>)	401
Algunas notas sobre las plantas medicinales, aplicadas en Cuba (<u>C. R. Martínez Callís, M. Fernández Zequeira, R. Oviedo Prieto</u>)'	411
BOOK REVIEWS (ed.: <u>K. T. Kiss</u>)	
Borhidi, A.: Phytogeography and Vegetation Ecology of Cuba (<u>Z. Kereszty</u>)	417
Natho, G.—Müller, C.—Schmidt, H.: Morphologie und Systematik der Pflanzen I—II (<u>Z. Kereszty</u>)	419
Cullen, J.—Davis, P. H.: The identification of flowering plant families (<u>Z. Kereszty</u>) ..	420
Gledhill, D.: The names of plants (<u>Z. Kereszty</u>)	420
Weberling, F.: Morphology of flowers and inflorescences (<u>Z. Kereszty</u>)	421
Delcourt, P. A.—Delcourt, H. R.: Long-term Forest Dynamics of the Temperate Zone (<u>M. Járαι-Komlódi</u>)	422
Thrower, S. L.: Hong-Kong Lichens (<u>E. Farkas</u>)	422
Hindák, F.: Studies on the Chlorococcal algae (<u>Chlorophyceae</u>) V (<u>É. Ács</u>)	423
Callow, J. A. (ed.): Advances in Botanical Research. Vol. 14 (<u>B. Papp</u>)	423
Fukuyo, Y.—Takano, H.—Chihara, M.—Matsuoka, K. (eds): Red Tide Organisms in Japan (<u>K. T. Kiss</u>)	424
Popovsky, J.—Pfiester, L. A.: Dinophyceae (Dinoflagellida) (<u>K. T. Kiss</u>)	425

CONTRIBUTION TO THE STUDY OF STREPTOMYCES VIOLASCENS FROM THE AUSTRALIAN FOREST ENVIRONMENT

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(Received: 16 March 1992)

Seven isolates of actinomycetes from different forest habitats in New South Wales were described and identified as Streptomyces violascens though they exhibited different degrees of taxonomic proximity to this taxon. One of these organisms originated a mutant on agar which, in turn, was found to be very similar to a streptomycete from different sources, under the experimental conditions.

Introduction

In 1931 JENSEN published an account of the actinomycetes from a number of soils in Australia. It included a description of seventeen species of the genus Actinomyces (renamed Streptomyces by WAKSMAN and HENRICI 1943) among which Actinomyces (Streptomyces) albus, A. griseus and A. hygroscopicus.*

A long period of neglect in actinomycetes studies in Australia ensued JENSEN's return to Europe in the fifties.

During a long-term investigation on the actinomycetes population of certain forest sites initiated in the eighties in New South Wales, with particular reference to the genera Streptomyces, Streptoverticillium and Chainia, a streptomycete characterized by a purple sporogenous aerial mycelium (formed later, as it will be explained) was often recovered. On one occasion, up to 46 per cent (65 out of 142) of the colonies randomly selected from the plates of isolation from soil, resulted in belonging to this taxon. An in-depth study was thus initiated with similar isolates from different habitats in this State. A variant, originating in a culture of this micro-organism on mineral agar 1 of GAUZE et al. (1957), was also studied

*S. hygroscopicus and related species have frequently been recovered in New South Wales (GERRETTSON-CORNELL 1983-84, 1987-88; unpublished data).

on a comparative basis with its parent strain and a different isolate from the author's collection.

Material and Methods

Origin of the field strains of actinomycetes

The origin of the isolates of this survey, except the mutant which is described separately, is shown in Table 1.

Table 1

List of isolates of actinomycetes

Strain No.	Area of origin	Host
1	Tumut, N.S.W.	Soil from a 2-year-old <u>Pinus radiata</u> plantation on formerly pasture site
2	Tumut, N.S.W.	Soil from a 2-year-old <u>Pinus radiata</u> plantation on formerly pasture site
M 1	Sunny Corner State Forest (Bathurst) N.S.W.	Rhizosphere of 2-year-old <u>P. radiata</u> trees on a formerly hardwood* site
N 4	Sunny Corner State Forest (Bathurst) N.S.W.	Rhizosphere of 2-year-old <u>P. radiata</u> trees on a formerly pasture site
B 34	Engadine, N.S.W.	Rhizosphere of <u>Casuarina glauca</u>
G 14	Cumberland National Forest (West Pennant Hills) N.S.W.	Rhizosphere of <u>C. cunninghamiana</u>
Cn 5	Canobolas Nursery (Orange) N.S.W.	Soil under 1-year-old <u>P. radiata</u> seedlings

*A mixture of Eucalyptus fastigiata, E. dalrympleana and E. pauciflora.

Isolation, characterization and identification of the field actinomycetes

The actinomycetes of the present study were obtained in the course of isolation tests directly from soil or from the thin layer of soil adherent to the root surface. The isolation from soil was carried out as indicated in a previous work (GERRETTSON-CORNELL and KELLY 1981). For the isolation from the root surface (rhizoplane-rhizosphere), a technique previously described (GERRETTSON-CORNELL 1983-84) was also used except that 3 g of root pieces instead of 10 g were suspended in sterile deionized glass distilled water. The isolates of the present study were characterized and identified by traditional taxonomic methods rather than by current numerical schemes (WILLIAMS *et al.* 1983; WILLIAMS *et al.* 1989). Dismissing all work carried out in the past is in the author's opinion unjustified. Moreover, in spite of certain limitations and the necessity for improvement, the traditional approach to classification can still play an important role. In this particular case, it has proved quite adequate.

Characterization of isolates was thus based on the methodology of the International Streptomyces Project (ISP) (SHIRLING and GOTTLIEB 1966) supplemented with the use of Czapek's agar, nutrient agar, potato plugs and mineral agar 1 of GAUZE *et al.* (1957). Production of

H₂S, the ability to hydrolize starch, gelatin and casein, and finally nitrate reduction were also tested.

The spore wall morphology was observed on impression mounts with a transmission electron microscope in cultures on ISP media 3 and 4. The Methuen Handbook of Colour (KORNERUP and WANSCHER 1963) was occasionally used to delineate colour instead of the Tresner and Backus colour wheels whose publication was discontinued many years ago.

A preliminary identification was carried out using a computerized key (GERRETSON-CORNELL and GWALTER 1985) based on the ISP's descriptions of 465 taxa (SHIRLING and GOTTLIEB 1968a, b, 1969, 1972). The results of this exercise were compared with the schemes of PRIDHAM and TRESNER (1974), SZABÓ *et al.* (1975), GAUZE *et al.* (1983) and with the descriptions of taxa (GAUZE *et al.* 1957; GAUZE *et al.* 1983; SHIRLING and GOTTLIEB 1968b).

Detection, isolation and characterization of the mutant

A 4-cm long section of a six-week-old culture of strain M1 on agar medium 1 was found to differ markedly from the rest of the colony. It produced a reddish-purple endopigment which, however, did not diffuse through the surrounding medium and spore chains all rectiflexibles. Conversely, the parent culture showed no endopigment and sporophores of the spirales and retinaculumapertum type. The demarcation line between these two zones was distinct. To assess the extent of this variation, two groups of tests were effected.

The first group consisted of sub-culturing the mycelium from either side of the colony (mutant and adjacent parent culture) on to ISP medium 2 slants and then using these fresh cultures as a source of inoculum in comparative tests between the parent strain and the mutant.

The second group of experiments was based on monosporial isolations from each side of the original culture. Thus, seven cultures were later randomly selected from the monosporial isolation plates of the parent strain and seven from the mutant's.

Characterization of isolates followed the same procedure as for the field isolates of actinomycetes.

Results and Discussion

Characterization and identification of the field isolates of actinomycetes

The cultural, morphological and physiological features of these actinomycetes are collated in Tables 2 to 6. All strains exhibited a peculiar phenomenon when firstly isolated. They failed to produce the aerial mycelium for as long as 6-10 weeks. Sometimes it took three consecutive transplants for a few, puny islands of white, sporigenous aerial mycelium to be formed. These eventually merged into larger patches and then into a uniform mat which turned gradually to purple. Once acquired, the ability to yield aerial mycelium was never lost. All strains exhibited, at maturity:

- a spiny spore wall. Spores were approximately cylindrical and measured 0.7-1.0 μm x 0.4-0.6 μm ;
- a prevailing spiral type of spore chain, coiled or extended. The retinaculumapertum type was also found to be present;
- a sporigenous aerial mycelium, purple on more than one medium;
- a positive melanin reaction on ISP medium 6 and weak to positive on ISP medium 7.

Table 2

Melanin reaction and H₂S formation by the actinomycetes

Strain No.	Melanin reaction			H ₂ S
	M 1	M 6	M 7	
1	$\pm, +$	$-, +$	$\pm, +$	-
2	$\pm, +$	+	$\pm, +$	+
M 1	+	+	$\pm, +$	$-, \pm$
N 4	-	+	+	-
B 34	-	+	\pm	$\pm, +$
G 14	+	+	$\pm, +$	+
CN 5	$\pm, +$	+	$-, \pm$	$\pm, +$

M 1, M 6, M 7: ISP Media 1, 6, 7

-: Nil

 \pm : Weak

+: Strong

Table 3

Diastatic activity, proteolytic activity and nitrate reduction of the actinomycetes

Strain No.	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	Nitrate reduction
1	+	-	-	$\pm, +$
2	++	$-, \pm$	-	-
M 1	$\pm, +$	-	-	-
N 4	+	-	-	$\pm, +$
B 34	++	\pm	++	++
G 14	++	\pm	\pm	\pm
CN 15	+	-	-	$-, \pm$

-: less than 3 mm wide enzymatic zone

 \pm : between 3 and 5 mm wide enzymatic zone

+: equal to or over 5 mm but less than 10 mm wide enzymatic zone

++: equal to or over 10 mm wide enzymatic zone

This set of features matched the description of *Streptomyces violascens* (PREOBRAZHENSAYA and SVESHNIKOVA) PRIDHAM, HESSELTINE and BENEDICT (GAUZE *et al.* 1957; GAUZE *et al.* 1983; SHIRLING and GOTTLIEB 1968b), an actinomycete originally isolated in Russia from soil samples received from Uruguay, other South American countries and South Africa (KALAKOUTSKII's pers. com.). Results of the carbon utilization test generally confirmed this

Table 4
Carbon utilization test of the actinomycetes

Strain No.	G	S	X	I	M	F	Ra	Rf	A
1	+	-	+	±	-	+	-	+	+
2	±	+	±,+	+	-,±	+	-,±	+	+
M 1	+	-,±	±,+	+	±	+	-,±	+	+
N 4	+	-	+	+	+	+	+	+	+
B 34	+	±	±,+	+	-	+	+	±,+	+
G 14	+	±,+	-,±	±,+	-,±	+	±	±,+	+
CN 5	+	-	±,+	+	+	±,+	±,+	+	+

G: D-glucose S: sucrose X: D-xylose I: I-inositol
 M: D-mannitol F: D-fructose Ra: rhamnose Rf: raffinose
 A: L-arabinose

-: none ±: weak or doubtful +: strong

identification through the use of our key and PRIDHAM and TRESNER's scheme. When the strictly dichotomic key of SZABÚ *et al.* (1975) was employed, those strains which could not utilize rhamnose and mannitol resulted conspecific with Actinomyces* (Streptomyces) violascens. On the other hand, even for those isolates which did utilize these two carbon sources, this identification represented the only alternative.

Further comparison with the original description of S. violascens showed a nitrate reduction to be moderate by this taxon and weak to moderate by our strains (except B34** for which it was found to be very strong). Hydrolysis of gelatin by our isolates was practically identical to that of S. violascens. Alternatively, on potato plugs and nutrient agar, the strains of this study exhibited a closer similarity to that Actinomyces (Streptomyces) lavendulae described by WAKSMAN and CURTIS (1916) which is quite distinct from the neotype of it established several decades later. Like the original "lavendulae" in fact, our strains released a brown to black pigment on the above media (Table 6).

*Actinomyces sensu KRASSILNIKOV.

**Initially identified by the writer as in between S. violaceus and S. violascens, but closer to the latter, was later re-identified by Dr. KALAKOUTSKII of the Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Science, Moscow, as S. violascens.

Table 5

Cultural and morphological characteristics of the actinomycetes on ISP media 2, 3, 4 and 5

Strain No.	Medium of cultivation	Description
1	2	Am white turning to pale 'cream' and then to purple. Rev. 'camel' (6C4-6D4) or brown with a hue of yellow. No s.p. or light yellowish-brown. Spore chain: S (extended)
	3	Am lanose, scanty, light purple. Ref. Colourless. No s.p. Spore chain: S (extended)
	4	Am lanose, lilac. Rev. beige. No s.p. Spore chain: S (extended)
	5	Am lanose, lilac with some white areas. Rev. colourless to yellowish beige. No s.p. Spore chain: S (extended)
2	2	Am lanose, flat, greyish purple to purple. Colourless to yellowish exudate produced. Rev. brown (6E4). No s.p. or traces of yellow-brown. Spore chain: RA, S (compact, more seldom extended or irregular)
	3	Am lanose, thin, light purple. Rev. colourless. No s.p. Spore chain: RA, S (coiled or irregular)
	4	Am lanose, flat, light beige with a purple hue. Rev. light beige or grey-brownish. No s.p. or pale brown. Spore chain: RA, S (coiled)
	5	Am lanose, purple. Rev. grey-brown. No s.p. Spore chain: S (extended), RA
M 1	2	Am lanose, flat, white turning to 'cream' and then to greyish-purple or purple. Colourless or yellowish exudate produced. Rev. tan or leather brown (6E6). No s.p. or light yellow-brown. Spore chain: S (coiled or irregular), RA
	3	Am lanose, purple. Rev. colourless. No s.p. Spore chain: S (extended)
	4	Am lanose, greyish-purple to purple. Rev. deep grey with a hue of purple. Grey s.p. round the culture. Spore chain: S (extended)
	5	Am lanose, white to purple. Rev. beige. No s.p. Spore chain: S (extended)
N 4	2	Am lanose, flat, light 'cream' turning to greyish-purple and then to purple. Yellowish or colourless exudate. Rev. light brown to brown. Yellowish-brown or no s.p. Spore chain: S (irregular or coiled), RA
	3	Am lanose, thin, purple. Rev. colourless. No s.p. Spore chain: S (extended or compact)
	4	Am lanose, light 'cream' turning to greyish-purple and then to purple. Rev. beige. Brownish-grey with a hue of purple s.p. Spore chain: S (extended)
	5	Am thin, lanose, white. Rev. beige. No s.p. Spore chain: S (extended)

Table 5 (cont.)

Strain No.	Medium of cultivation	Description
B 34	2	Am lanose, purple. Rev. light beige. No s.p. Spore chain: S (extended)
	3	Am lanose, lilac. Rev. beige. No s.p. Spore chain: S (extended)
	4	Am lanose, light purple. Rev. beige. No s.p. Spore chain: S (extended)
	5	Am lanose, white. Rev. colourless to beige. No s.p. Spore chain: S (extended)
G 14	2	Am lanose, light purple. Rev. light brown. No s.p. Spore chain: RA, S (extended or coiled)
	3	Am lanose, white. Rev. colourless. No s.p. Spore chain: S (extended)
	4	Am light purple. Rev. beige. No s.p. Spore chain: S (extended or coiled)
CN 5	5	Am lanose, white. Rev. colourless. No s.p. Spore chain: S (extended or coiled)
	2	Am lanose, white turning to light purple. Rev. beige. No s.p. Spore chain: S (extended)
	3	Am in puny islands, lanose, white. Rev. colourless. No s.p. Spore chain: S (extended)
	4	Am <u>lilac</u> -purple. Light beige reverse. No s.p. Spore chain: S (extended)
	5	Am lanose, white. Rev. colourless. No s.p. Spore chain: S (extended)

Am: aerial mycelium
S: spirales

Rev.: reverse
RA: retinaculum apertum

s.p.: soluble pigment

Though there is no comparative description under standard conditions of S. violascens and WAKSMAN and CURTIS' A. lavendulae, the similarity of these two organisms is striking. So much so that PRIDHAM et al. (1965) wrote: "Probably, living material representing the true S. lavendulae now is known as Streptomyces violascens (PREOBRAZHENSKAYA and SVESHNIKOVA) PRIDHAM, HESSELTINE and BENEDICT (1958). The spores are spinose and occur in coiled chains. Also, the aerial mycelium exhibits colours more definitely characterized as 'lavender or violet' than do strains now labelled lavendulae." A literature review on this aspect is now in progress and will soon be submitted for publication.

Table 6

Cultural and morphological characteristics of the actinomycetes on Czapek's agar (Cz), nutrient agar (NA), potato plugs (PP) and mineral agar 1 (MA 1)

Strain No.	CZ	NA	PP	MA 1
1	Am: thin, white Rv: colourless Sp: none Sc: S (ext.)	none colourless brown not app.	none yellowish black not app.	thin, white beige none S (ext.)
2	Am: ibid. Rv: ibid. Sp: ibid. Sc: ibid.	ibid. ibid. ibid. ibid.	ibid. ibid. ibid. ibid.	very scarce colourless none
M 1	Am: ibid. Rv: ibid. Sp: ibid. Sc: ibid.	ibid. ibid. ibid. ibid.	ibid. ibid. ibid. ibid.	lanose, lilac colourless none S (ext. or coiled)
N 4	Am: ibid. Rv: ibid. Sp: ibid. Sc: ibid.	ibid. ibid. ibid. ibid.	ibid. ibid. ibid. ibid.	lanose, white beige none RF
B 34	Am: ibid. Rv: ibid. Sp: ibid. Sc: ibid.	ibid. ibid. ibid. ibid.	ibid. ibid. ibid. ibid.	thin, white colourless none S (coiled), RA
G 14	Am: thin, lilac Rv: colourless Sp: none Sc: S (ext.)	ibid. ibid. ibid. ibid.	ibid. ibid. ibid. ibid.	thin, white colourless none S (ext.)
CN 5	Am: ibid. Rv: ibid. Sp: ibid. Sc: ibid.	ibid. ibid. ibid. ibid.	ibid. ibid. ibid. ibid.	thin, white colourless none S (ext.)

Am: aerial sporigenous mycelium

Rv: reverse colour

Sp: soluble pigment

Sc: spore chain type

Not app.: not applicable due to insufficient or no development of the aerial mycelium.

S: spirales

RA: retinaculum apertum

RF: rectiflexibiles

ext.: extended

As previously indicated, there was a certain degree of diversity between and within some of the isolates of this investigation. For instance, two of the seven replicates of strain No. 1 from Tumut, on ISP medium 6 resulted melanin negative. Strains N4 and B34 proved melanin negative on ISP medium 1, differently from the other strains. On ISP medium 4, the sporigenous aerial mycelium of strains CN5 was lilac-purple, quite distinct from the purple mycelium of strains M1 and N4. On ISP medium 2 on the other hand,

there was practically no difference in colour between CN5 and the other two isolates; however the reverse of M1 and N4 was much darker than that of CN5. Differences also existed between strains 1 and 2 from Tumut with regard to H_2S formation, nitrate reduction and utilization of sucrose. Finally, the overall similarity with S. violascens varied from moderate for CN5 to exceptionally good in strains 1 and M1. It was considered good in the other strains; no alternative identification could be envisaged.

Finally, it is noteworthy that WILLIAMS et al.'s (1989) scheme refers to S. violascens as a 'subjective synonym' of S. violaceus (ROSSI-DORIA) WAKSMAN. In their work, S. violaceus is listed as a cluster species encompassing S. violaceus, S. cellostaticus and S. violascens at the 91 per cent S-level and a few other species at a lesser degree. In WILLIAMS et al.'s work, the above three species are reported as having spiral chains of spiny, violet spores. Conversely, participants in the ISP described S. violaceus as having a light yellowish pink or greyish yellowish pink (Red series) sporigenous aerial mycelium.

Characterization of the mutant

Cultures obtained by directly streaking the mycelium from the parent strain or the mutant on to fresh agar (first group of tests) showed no differences with their respective sources.

In the second group of experiments, four of the seven cultures obtained by monosporial isolation from the parent strain appeared unchanged, two were changing (having produced the above reddish-purple endopigment on two media) and one appeared like an exact replica of the mutant. By contrast, the seven monosporial isolations from the mutant showed no differences to it.

For about five months since the mutation in strain M1 was first noticed, colonies derived from it seemed quite stable. Thereafter, the sporigenous aerial mycelium of these on ISP media 4 and 5 produced areas of purple colour, this being more marked on medium 5. The main features of the mutant, to be compared with its parent strain's (M1) description in Tables 2 to 6, are listed hereunder.

-- On ISP medium 2: aerial sporigenous mycelium velvety-lanose, pale greyish-yellowish (Yellow series); vegetative mycelium and reverse brown-blackish or dark brown in the centre, with or without a purplish hue in certain parts. No soluble pigment produced. Spore chain type: rectiflexibiles (also in tufts) only.

-- On ISP medium 3: aerial sporigenous mycelium velvety-lanose, very pale greyish-yellowish (Yellow series) or pale yellow (Yellow series); vegetative mycelium and reverse reddish-purple; no soluble pigment formed. Spore chain type: rectiflexibiles (also in tufts) only.

-- On ISP medium 4: aerial sporigenous mycelium velvety-lanose, pale greyish-yellowish or greyish-greenish-yellowish (Yellow or Green series), purplish in certain areas. Vegetative mycelium and reverse reddish-purple to purple. Some greyish-pinkish soluble pigment around the colony. Spore chain type: rectiflexibiles (also in tufts) only.

-- On ISP medium 5: as on medium 4, except for a more extended purplish colour of the aerial mycelium.

-- On Mineral Agar 1: aerial sporigenous mycelium velvety-lanose, "whitish". Reverse and vegetative mycelium reddish-purple. No soluble pigment. Spore chain type: rectiflexibiles only.

-- On potato plugs: aerial sporigenous mycelium whitish to light purple. Vegetative mycelium reddish-purple. Grey soluble pigment.

-- On Czapek's agar: aerial sporigenous mycelium very thin, lanose, white. Reverse colourless. No soluble pigment. Spore chain type: rectiflexibiles only.

-- Spore surface ornamentation on ISP media 3 and 4: smooth.

-- Melanin reaction on ISP media, 1, 6 and 7: negative.

-- H₂S formation on ISP medium 6: very strong.

-- Nitrate reduction: variable.

-- Diastatic activity: strong.

-- Proteolytic activity (gelatin and casein liquefaction): strong.

-- Carbon sources utilization (SHIRLING and GOTTLIEB 1966): unlike its parent strain M1, the mutant could not utilize inositol and arabinose. No difference with regard to the other seven carbon sources.

Based on these features and properties, the difference between mutant and parent strain can be defined as striking. The spore wall which changed from spiny (long spines) to smooth, the spore chain type from spiral to rectiflexibiles and the melanin reaction from positive to negative are just a few examples of this variation.

Finally, an exceptionally close similarity if not identify (since no examination of the genomes could be carried out) was shown, under the experimental conditions, between our mutant and a streptomycete isolated from different natural habitats in New South Wales. The latter had originally been identified as a possible variant of S. griseus according to SCHATZ and

WAKSMAN (1945) and WAKSMAN (1961: 141). The problem is an interesting one but quite complex. It adds evidence to the hypothesis that we may at times be identifying mutants directly from soil. On the subject of mutation in actinomycetes, there is an extensive literature. For instance, BALDACCI attributed great importance to mutants in Streptomyces. He wrote, in a joint paper (BALDACCI et al. 1963): "It is desirable that, in future strains obtained by spontaneous or induced mutation, should be listed as such and, even though by using autonomous criteria, classified within the known genera and species isolated from nature." And again (BALDACCI 1982--83): "Classification based on what is found in nature could be called 'natural'. Artificially obtained mutations should constitute a sub-division in classification. ... The mutated species could be catalogued in the same way and, if then found to be useful, they could be patented."

While writing this paper, two additional isolates of S. violascens have been recovered from the rhizosphere of P. radiata trees in this State which adds further evidence to the theory of the widespread distribution of this species in our forests.

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This modest contribution is devoted to the memory of the late Emeritus Prof. E. BALDACCI.

REFERENCES

- Baldacci, E., Locci, R., Farina, G., Vegetti, G. (1963): Sulla mutabilità indotta di alcuni caratteri di Streptomyces utilizzati nelle classificazioni. Gior. Microb. 11: 137-166.
- Baldacci, E. (1982-83): My life with actinomycetes. The Actinomycetes 17: 3-45.
- Gauze, G. F., Preobrazhenskaya, J. P., Kudrina, E. S., Blinov, N. O., Ryabova, I. D., Sveshnikova, M. A. (1957): Problems in the classification of antagonistic actinomycetes. State Publishing House for Medical Literature. Medgiz, Moscow (English), 27-30.
- Gauze, G. F., Preobrazhenskaya, T. P., Sveshnikova, M. A., Terekova, L. P., Maximova, T. S. (1983): A guide to the identification of actinomycetes (genera Streptomyces, Streptovorticillium and Chainia). Scientific Publishing House "Nauka", Moscow, 122-126 (Russian).
- Gerrettson-Cornell, L. (1983-84): Note on some actinomycetes from the rhizosphere of Casuarina littoralis Salisb. and their antagonistic relationship with P. cinnamomi, in vitro. The Actinomycetes (USA) 18: 78-99.

- Gerrettson-Cornell, L. (1987-88): Studies of some actinomycetes from the root zone of Allo-casuarina verticillata (Lam.) L. Johnson. The Actinomycetes (USA) 20: 60-80.
- Gerrettson-Cornell, L., Gwalter, J. (1985): A numerical key for the identification of Streptomyces and Streptoverticillium included in the International Streptomyces Project. For. Comm. N.S.W. Tech. paper No. 31. 155 pp.
- Gerrettson-Cornell, L., Kelly, J. (1981): Note on the actinomycetes of a forest soil in New South Wales. 1. Isolation and preliminary characterization. For. Comm. N.S.W. Res. Note No. 46. 22 pp.
- Jensen, H. L. (1931): Contribution to our knowledge of the actinomycetes. II. The definition and sub-division of the genus Actinomycetes with a preliminary account of Australian soil actinomycetes. Proc. Linn. Soc. N.S.W. 56: 354-370.
- Kornerup, A., Wanscher, J. H. (1963): Methuen Handbook of Colour. Methuen and Co., London, 223 pp.
- Pridham, T. G., Tresner, H. D. (1974): Streptomycetaceae Waksman and Henrici, 1943, 339. In: Buchanan, R. E., Gibbons, N. E. (eds): Bergey's Manual of Determinative Bacteriology, 8th Edition. The Williams and Wilkins Co., Baltimore. 747-829.
- Pridham, T. G., Lyons, A. J., Seckinger, H. L. (1965): Comparison of some dried holotype and neotype specimens of streptomycetes with their living counterparts. Intern. Bull. Bact. Nom. and Taxon. 15: 191-237.
- Schatz, A., Waksman, S. A. (1945): Strain specificity and production of antibiotic substances. IV. Variations among actinomycetes, with special reference to Actinomycetes griseus. Proc. Nat. Acad. Sci. U.S. 31: 129-137.
- Shirling, E. B., Gottlieb, D. (1966): Methods for characterization of Streptomyces species. Int. J. Syst. Bact. 16: 313-340.
- Shirling, E. B., Gottlieb, D. (1968a): Co-operative description of type cultures of Streptomyces. II. Species description from first study. Int. J. Syst. Bact. 18: 69-189.
- Shirling, E. B., Gottlieb, D. (1968b): Co-operative description of type cultures of Streptomyces. III. Additional species descriptions from first and second studies. Int. J. Syst. Bact. 18: 270-392.
- Shirling, E. B., Gottlieb, D. (1969): Co-operative description of type cultures of Streptomyces. IV. Species description from the second, third and fourth studies. Int. J. Syst. Bact. 19: 391-512.
- Shirling, E. B., Gottlieb, D. (1972): Co-operative description of type strains of Streptomyces. V. Additional descriptions. Int. J. Syst. Bact. 22: 265-394.
- Szabó, I. M., Marton, M., Buti, I., Fernandez, C. (1975): A diagnostic key for the identification of species of Streptomyces and Streptoverticillium included in the International Streptomyces Project. Acta Bot. Acad. Sci. Hung. 21: 387-418.
- Waksman, S. A., Curtis, E. R. (1916): The actinomycetes of the soil. Soil. Sci. 1: 99-134.
- Waksman, S. A. (1919): Cultural studies of species of actinomycetes. Soil Sci. 8: 71-215.
- Waksman, S. A. (1961): The Actinomycetes II. Classification, identification and description of genera and species. The Williams and Wilkins Co., Baltimore, 141 pp.
- Waksman, S. A., Henrici, A. T. (1943): The nomenclature and classification of actinomycetes. J. Bact. 46: 337-341.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A., Sackin, M. J. (1983): Numerical classification of Streptomyces and related genera. J. Gen. Microb. 129: 1743-1813.
- Williams, S. T., Goodfellow, M., Alderson, G. (1989): In: Bergey's Manual of Systematic Bacteriology, Vol. 4. Williams and Wilkins.

STUDIES ON THE POWDERY MILDEW FUNGUS, *SPHAEROTHECA FULIGINEA*: ULTRASTRUCTURE AND HOST RESPONSES

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According to the criteria of conidia and cleistothecia, this study revealed that the powdery mildew fungus which infects squash in Nile Delta, Egypt is *Sphaerotheca fuliginea*. This fungus was studied by light microscopy, scanning and transmission EM. The conidia are barrel or oval-shaped with wrinkled surface. The cleistothecia are spherical and contain one ellipsoid to spherical ascus with 4-8 ellipsoid ascospores. The host epidermal cell was penetrated by a penetration peg which, after penetration developed into haustorium consisting of a nucleated central body with several lobes, embedded in a matrix. The surrounding extrahaustorial membrane is an invagination of the plasmalemma of the host cell. As a defence mechanism, a collar was formed around the penetration peg. Chloroplasts of infected leaf of squash showed dramatic changes in their ultrastructure. The infection caused an alteration in the physiology of squash leaf. It is obvious that there was a marked depletion in reducing sugars and sucrose which accompanied by a simultaneous elevation in polysaccharides of infected leaf. Also, the pigment content (chl.a, b and carotenoids) appeared to be reduced as a result of such infection. There was a massive decrease in the endogenous growth stimulant levels (auxins, gibberellins and cytokinins) with a concomitant increase in growth inhibitors in infected leaf of squash.

Keywords: physiology, *Sphaerotheca fuliginea*, squash, ultrastructure

Introduction

Powdery mildew of cucurbits is a serious disease and common in Nile Delta of Egypt. It occurs on squash (*Cucurbita pepo* L.), cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.) and watermelon (*Citrullus vulgaris* Schrad.). The causal organism is still a subject of debate. Both *Erysiphe cichoracearum* DC. ex Merat. emend Salmon and *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. have been implicated as causal agents of the disease. Since the two pathogens are similar in their conidial stage, it is difficult to distinguish between them in the absence of the sexual stage. BALLANTYNE (1975) stated that *S. fuliginea* is the predominant species on cucurbits in some countries and probably the only species in others. Several other workers have also observed the dominance of *S. fuliginea* in powdery

mildew of cucurbits (GORTER 1966; SIRADHANA and GHANDHARI 1972; SOKOLOV 1978). Otherwise, S. fuliginea has been identified as a causal agent of powdery mildew of cucurbits on the basis of cleistothecial characteristics (REIFSCHNEIDER et al. 1985; IBRAHIM et al. 1985; LETHAM and PRIEST 1989). Recently and according to the criteria of the conidial stage, AWAD et al. (1990) reported that S. fuliginea is a causal organism of cucurbits in Egypt.

In all powdery mildew haustoria examined by electron microscopy (BRACKER and LITTLEFIELD 1973; EHRLICH and EHRLICH 1963, 1971; GIL and GAY 1977; EBRAHIM-NESBAT et al. 1986; EDWARDS and ALLEN 1970; HIRATA and KOJIMA 1962; PERERA and GAY 1976), the haustorium and its lobes lie in a pocket of membrane (the extrahaustorial membrane) which is almost certainly an invagination of the plasma membrane of the host cell (BUSHNELL and GAY 1978). In this connection, still little informations about the ultrastructure of S. fuliginea on squash.

Powdery mildews as most of biotrophic fungi caused metabolic changes in the leaf tissue of the host. Infection by biotrophic fungi may lead to substantial changes in the carbohydrate content of infected plants which may reflect the alteration in the different metabolic processes favourable or unfavourable for fungal development (HWANG and HEITEFUSS 1986). JINDAL et al. (1979) and KABASCH (1982) observed a reduction of soluble sugars in cucumber leaves infected with S. fuliginea. In addition, powdery mildews may diminish the rate of photosynthesis by affecting either the chloroplasts or chlorophyll content directly or through the enzymes concerned with photosynthesis (BUSHNELL and ALLEN 1962). Infection by biotrophic fungi greatly alter the endogenous hormonal levels in infected leaf tissues. BUSHNELL (1984) reported that rust fungi modify infected tissues either by producing the hormones themselves or by changing the local concentrations of host-produced hormones.

The objectives of this study are to add more informations at the ultrastructural level about S. fuliginea on squash and the effect of this fungus on some physiological aspects of the host.

Material and Methods

Microscopy

Light microscopy (LM). Infected leaves of squash (Cucurbita pepo L.) by Sphaerotheca fuliginea (Schlecht. ex Fr.) Poll. were collected from the field at Baltim area, North Delta for the present investigation. To detect the number of asci inside the cleistothecium, pieces of old infected leaf were prefixed in Formaline-Acetic acid-Alcohol (3:1:1), dehydrated in graded series of ethanal, embedded in wax and sectioned according to the method of JENSEN

(1962). In addition, semi-thin sections ($0.5\ \mu\text{m}$) were prepared for LM examination from Araldite-embedded tissues as the following procedure.

Transmission electron microscopy (TEM). Infected and healthy leaves were processed for TEM by a method based on WOODS and GAY (1987). Leaf pieces ($1.0\ \text{mm}^2$) were prefixed in 2.5% glutaraldehyde in 1.0 M cacodylate buffer at pH 7.0. Then, the leaf pieces were postfixed in 1.0% osmium tetroxide in the same buffer, dehydrated in graded series of ethanol and embedded in Araldite. Ultrathin sections were cut using Reichert ultramicrotome, stained by 2.0% aqueous uranyl acetate followed by lead citrate (REYNOLDS 1963). The sections were viewed and photographed using Phillips 301 TEM.

Scanning electron microscopy (SEM). This method adapted from MERCER and BIRBECK (1972). Infected leaf segments at conidial stage were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.0. The segments were dehydrated through ethanol series and then passed in acetone series. The specimens were dried using critical point drying apparatus and coated with gold. The specimens were then examined and photographed using Phillips SEM-501.

Physiological aspects

Relative water content. The relative water content of both healthy and infected leaves of squash was determined according to the technique described by WEATHERLY (1950).

Estimation of pigments. The photosynthetic pigments (chl.a, b and carotenoids) of healthy and infected leaves were estimated according to spectrophotometric method recommended by METZNER *et al.* (1965).

Estimation of carbohydrates. The method of extraction and clarification of carbohydrates from healthy and infected leaves were essentially those described by YOUNIS *et al.* (1969). The direct reducing value (D.R.V.) which was considered to be equivalent to reducing sugars was determined following the procedure of NELSON (BELL 1955). The total reducing value (T.R.V.) was estimated by determining the optical density after hydrolysis by invertase. The sucrose content was calculated from the difference between T.R.V. and D.R.V. Polysaccharides were determined in the dry residue after alcohol extraction as adopted by YOUNIS *et al.* (1969).

Hormone extraction, purification and bioassay. The method of extraction was that originally described by SHINDY and SMITH (1975). For determination of abscisic acid in extracts, wheat coleoptile bioassay developed and adopted by WRIGHT (1956) was used. The amount of either acidic or neutral auxins was estimated according to straight growth test of barley coleoptile adopted by FODA and RADWAN (1962). Gibberellic acid in extracts was determined by the lettuce hypocotyl bioassay developed and adopted by FRANKLAND and WAREING (1960). Cytokinin content of the extract was estimated according to the method described by ESASHI and LEOPOLD (1969).

Results

Microscopical observations

When infected leaves were examined by both LM and SEM, the mycelia were abundant and spreading on upper surface of infected leaf giving numerous conidiophores (Figs 1, 2 and 4). Also, the conidia were barrel or oval-shaped with wrinkled surface (Fig. 3). The conidia measured $32.2\text{--}38.9 \times 16.3\text{--}18.5\ \mu\text{m}$ and born as long chains. Cleistothecia were more or less spherical measured $75\text{--}100\ \mu\text{m}$. These cleistothecia have myceloid interwoven appendages which are variable in shape and size. The number of these appendages per cleistothecium ranged between 3-9 and their length between $60\text{--}250\ \mu\text{m}$. The cleistothecia have multilayered wall (Fig. 5) and contained one ellipsoid to spherical ascus, measuring $40\text{--}70 \times 35\text{--}55\ \mu\text{m}$ and with 4-8 ellip-

soid ascospores (Figs 6 and 7). Araldite-semithin sections revealed that infection hyphae were closely depressed to the surface of the epidermis of the host (Fig. 4). *Housteria* occurred abundant in the epidermal cells and occasionally two haustoria appeared in one epidermal cell (Fig. 4). No haustoria were observed in mesophyll cells. An electron micrograph depicted an electron-lucent zone in the epidermal wall around the infection peg (Fig. 8). The same micrograph showed a penetration peg enclosed by an electron-incent area, the collar. The matrix of this collar contains numerous vesicles (Fig. 8).

Cross-section of epidermal cell showed that haustoria were surrounded and separated from host protoplast by a single membrane (extrahaustorial membrane). The extrahaustorial matrix, a wide zone, lies between the extrahaustorial membrane and haustorial wall and contains many haustorial lobes. The haustorial body has a single nucleus and lacks a large vacuole but several ones are common. The cytoplasm is usually rich with ribosomes (Fig. 9).

Ultrastructure of chloroplasts

Electron microscopical examination revealed that the chloroplasts from healthy leaves of squash show well-developed membrane structure with very dense stacks of grana, intergranal lamellae, chloroplast-bounding membrane and few starch grains (Fig. 10). On the other hand, chloroplasts of infected leaves show dramatic changes in their ultrastructure. They show breakdown of grana, disappearance of intergranal lamellae, disorganisation of bounding membrane and the increase in number of starch grains and plastoglobuli (Fig. 11).

Physiology of infected leaves

From the data presented in Table 1, it is clear that the pathogen causes a dramatic reduction in relative water content of infected leaf of squash. Therefore, an elevation in saturation water deficit is manifested in such leaf.

Concerning to the pigment content of infected leaf of squash, it is appeared to be reduced as a result of such infection during conidial stage, where there is a rapid decline in chl.a, chl.b, carotenoids and total pigments. On the other hand, chl. a/b ratio appeared to be increased in infected leaf if compared with the healthy one (Table 2).

It is obvious from Table 3 that there is a marked depletion in soluble sugars (reducing sugars and sucrose) accompanied by a simultaneous elevation

Table 1

Relative water content (R.W.C.) and saturation water deficit (S.W.D.) of healthy and infected leaves of squash

	R.W.C.	S.W.D.
Healthy	75.49 \pm 0.79	24.51 \pm 0.78
Infected	55.59 \pm 1.11	44.41 \pm 1.11

Table 2

Pigment content of healthy and infected leaves of squash ($\text{mg}\cdot\text{g}^{-1}\cdot\text{f.wt.}$)

	Chl.a	Chl.b	Chl.a/b	Carotenoids
Healthy	0.76 \pm 0.04	0.58 \pm 0.02	1.33 \pm 0.1	0.30 \pm 0.01
Infected	0.32 \pm 0.03	0.17 \pm 0.01	1.91 \pm 0.26	0.17 \pm 0.01

Table 3

Carbohydrate content of healthy and infected leaves of squash ($\text{mg}\cdot\text{g}^{-1}\cdot\text{d.wt.}$)

	Reducing sugars	Sucrose	Polysaccharides	Total carbohydrates
Healthy	41.66 \pm 0.57	73.15 \pm 0.95	86.11 \pm 0.42	200.92 \pm 1.81
Infected	2.34 \pm 0.32	18.03 \pm 0.52	160.15 \pm 1.39	180.52 \pm 2.07

Values are the mean of 4 samples of both healthy and infected leaves \pm standard error

in insoluble sugars of infected squash leaf. In general, the total carbohydrates decreased in infected leaf.

The data presented in Table 4 clearly elucidate that there is a massive decrease in the endogenous hormonal levels particularly the growth promotory substances (auxins, gibberellins and cytokinins) in infected leaf of squash. However, the growth inhibitory substances equivalent to abscisic acid was greatly as a consequence of infection. Also, the data revealed that the ratio of promoters: inhibitors is very low in infected leaf as compared to healthy one. In the infected leaf, the total amount of growth promoters, percentage concentration of each growth promotor related to the total promotor concentration is greatly changed as compared to healthy one. The per-

Table 4

Changes in endogenous hormonal levels ($\mu\text{g/g.f.wt.}$), percentage concentration of promotors and the ratios of promotors/inhibitors in healthy and infected leaf of squash

	Healthy	Infected
Auxins (IAA)	25.66 \pm 1.00	3.67 \pm 0.72
Auxins (%)	71.80	44.2
Gibberellins (GAs)	08.65 \pm 0.72	4.61 \pm 0.32
Gibberellins (%)	42.2	55.7
Cytokinins	01.44 \pm 0.25	0.03 \pm 0.008
Cytokinins (%)	04.02	00.38
Inhibitors (ABA)	05.28 \pm 1.23	08.28 \pm 1.00
Total promotors	35.75 \pm 2.72	08.32 \pm 2.12
Promotors/Inhibitors	06.68 : 1.0	1.0 : 1.0

Values are the mean of 4 samples of both healthy and infected leaves \pm standard error

centage of auxins represent the higher promotory one in healthy leaf, however, this growth promotory substance is drastically reduced in infected leaf and gibberellins percentage represent the higher growth promotory substance.

Discussion

According to cleistothecia observed and conidia, this study revealed that the causal agent for powdery mildew on squash and probably all cucurbits in Egypt is Sphaerotheca fuliginea and not Erysiphe cichoracearum. ROBERTS and BOOTHROYED (1989) stated that powdery mildew of cucurbits is caused by S. fuliginea, formerly called E. cichoracearum. The present work supported the observation of AWAD *et al.* (1990). They reported that S. fuliginea is the powdery mildew infected cucurbits in Egypt based on conidial criteria and not on cleistothecia. Other workers concluded that the powdery mildew of cucurbits is S. fuliginea (DEKHUIJZEN and VAN DER SCHEER 1969; KENIGSBUCH and COHEN 1989; COHEN *et al.* 1990). In the present study the measurements of conidia and cleistothecia differ than those of E. cichoracearum. In this connection, YARWOOD (1978) mentioned that conidia should usually not to be used in the taxonomy of Erysiphaceae. He added that shape and the presence of vacuoles, fibrosin bodies and oil drops within the

conidia seem to be their best diagnostic characters. However, we believe that the number of asci inside the cleistothecia is the most efficient criterion to distinguish between S. fuliginea and E. cichoracearum.

The appearance of an electron-lucent zone in host epidermal wall around the penetration peg may due to an interaction between enzymes produced by the fungus used in the penetration and materials of host wall. Based on electron microscope studies on powdery mildew on sunflower, McKEEN et al. (1966) suggested that changes in the epidermal host wall probably are due to enzyme(s) produced by the infection peg. Further cytochemical methods are needed to clarify this point.

The formation of collar around the penetration peg may be attributed to the defence mechanism of host cells against the invasion of the fungus. Callose seems to be the most frequent constituent of the collar (PRATT et al. 1984; SKOU 1985; SMART et al. 1986 a,b). Autofluorescence (HEATH and STUMPF 1986) indicates the presence of phenolic compounds similar to lignins (EDWARDS and AYRES 1981).

Basically, the ultrastructure of haustoria of S. fuliginea on squash resemble, in many respects, those of E. graminis on barley (BRACKER 1968), E. cichoracearum on sunflower (McKEEN et al. 1966), S. fuliginea on cucumber (DEKHUIJZEN and VAN DER SCHEER 1969), S. pannosa on rose (PERERA and GAY 1976) and L. taurica on green pepper (KUNOH et al. 1979).

The present investigation support the view that haustoria of S. fuliginea invaginate but do not penetrate the plasma membrane of epidermal cells of squash. We agree with the electron microscope observations of HIRATA and KOJIMA (1962) EHRLICH and EHRLICH (1963), BERLIN and BOWEN (1964), WOODS and GAY (1987) and BAKA (1987) on different biotrophic pathogens.

The present investigation revealed that the extrahaustorial matrix is stained more similar to epidermal wall than haustoria, this may give an indication that this matrix is host origin than fungal origin. This observation in agreement with the results obtained by many workers, SHAW and MANOCHA (1965) working on Puccinia graminis, BERLIN and BOWEN (1964) on Albugo candida, PEYTON and BOWEN (1963) on Peronospora manshurica, HIRATA (1937) on S. fuliginea, KOJIMA and HIRATA (1961) on Erysiphe graminis and BAKA (1987) on Puccinia punctiformis and P. lagenophorae. Further cytochemical studies may give more informations about the structure of S. fuliginea on cucurbits.

S. fuliginea appeared to modify the physiology of squash leaf tissue. Thus it is apparent that the infection by such provided biotroph caused a

marked depletion in chlorophyll content of leaf tissues. This depletion may be attributed to the change in ultrastructure of infected chloroplast or due to the senescence of infected tissue. BUSHNELL and GAY (1978) reported that when the fungus sporulates and makes its highest demands for food, host tissues at colony centres become chlorotic. ABOOD (1990) found that chlorophyll content of cucumber leaves infected by S. fuliginea was reduced to a great extent.

Although squash powdery mildew infection is restricted to epidermal cell, considerable changes occurred in the amount of ethanol soluble and insoluble carbohydrates in the leaf during the infection. The data showed that the level of soluble sugars decreased significantly in infected squash leaves when sporulation had started. Sucrose content in leaf tissues tended to be reduced by infection. The noticed decrease in reducing sugars and sucrose in infected tissue are likely to be due to demands of host tissues for energy and for biosynthetic intermediates for growth and sporulation of the pathogen (KOSUGE 1978). Also, during the sporulation sucrose is taking up from host photosynthate and converted to fungal metabolites (SMITH *et al.* 1969). There is some evidence to suggest that sucrose is the form of carbohydrate taken up by powdery mildew haustoria (HEWITT and AYRES 1976; MANNERS and GAY 1982). The present results revealed that polysaccharides which mainly starch increased very significantly in infected leaf. This is in coincidence with the increase of starch grains number in infected chloroplasts. Different host varieties may have different responses with respect to starch, following invasion by the same parasite (SCHIPPER and MIROCHA 1969) and different pathogens not always elicit the same type of host response (WHIPPS and COOKE 1977). The observed increase in polysaccharides content of infected leaves may presumably due to the enhancement production of abscisic acid which may induce the formation and accumulation of amylase inhibitors (MUNDY 1984). Starch content which represents the main pool of polysaccharides increased very significantly by powdery mildew infection. This pattern of changes in starch was in a good conformity with those obtained by MIROCHA and ZAKI (1966) and WHIPPS and COOKE (1978).

With regard to the changes in hormonal level of infected squash leaf tissues, it is appeared that there is a great depletion in growth stimulators accompanied by simultaneous accumulation of abscisic acid. Also, the ratio of promoters to inhibitors was obviously altered by infection. This variation in hormonal levels of host leaf tissues appeared to be in a close parallelism with other changes in host response due to the infection. This

phenomenon appeared to be clarified if we look to the changes in relative water content, chlorophyll and carbohydrate content of infected leaf of squash. The accumulative effect of abscisic acid in infected leaf may accelerate the induction of stress in infected tissues and this postulation was decisive when we noticed the depletion occurred in relative water content of such tissues. In addition, abscisic acid has been postulated to have an indirect role in promoting senescence in infected leaves (STODDART 1981).

It is evident from the obtained data that the decrease in gibberellins coincides with the accumulation of polysaccharides in infected leaves. In support of above-mentioned results, TAKAKI and DIETRICH (1980) proposed that polysaccharidase activities, enhanced by GAs. In addition, the role of cytokinins and auxins in the control of carbohydrates metabolism in infected leaves can be ruled out. Cytokinin may be substituted for gibberellin in the induction of amylase.

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REFERENCES

- Abood, J. K. (1990): Metabolic studies of powdery mildew infection of cucumber and its possible control by lithium chloride. Ph. D. Thesis. University of Sheffield, England.
- Awad, M. A., Mohammad, S. A., Khelifa, E. Z., El-Desouky, Sh. M., Elshanawani, M. Z. (1990): Etiology and physiology of cucurbit powdery mildew agent in Egypt. The 4th Internat. Mycol. Cong. Regensburg, Germany.
- Baka, Z. A. M. (1987): Responses of plant tissues to infection by rust fungi: Fine structure, cytochemistry and autoradiography. Ph. D. Thesis. University of Sheffield, England.
- Ballantyne, B. (1975): Powdery mildew on Cucurbitaceae: Identify, distribution, host range and sources of resistance. Proc. Linn. Soc. N.W. 99: 100-120.
- Berlin, J. D., Bowen, C. C. (1964): The host-parasite interface of Albugo candida in Raphanus sativus. Am. J. Bot. 51: 445-452.
- Bracker, C. E. (1968): Ultrastructure of the haustorial apparatus of Erysiphe graminis and its relationship to the epidermal cell of barley. Phytopathol. 58: 12-30.
- Bracker, C. E., Littlefield, L. J. (1973): Structural concepts of host-pathogen interfaces. In: Byrde, R. J. W., Cutting, C. V. (eds): Fungal Pathogenicity and the Plant Response. Acad. Press, London.
- Bushnell, W. R. (1984): Structural and physiological alterations in susceptible host tissue. In: Bushnell, W. R., Roelfs, A. B. (eds): The Cereal Rusts. I. Acad. Press, Inc.
- Bushnell, W. R., Allen, P. J. (1962): Induction of disease symptoms in barley by powdery mildew. Plant Physiol. 37: 50-59.

- Bushnell, W. R., Gay, J. L. (1978): Accumulation of solutes in relation to the structure and function of haustoria in powdery mildews. In: Spencer, D. M. (ed.): The Powdery Mildews. Acad. Press, London, N. Y., San Francisco, pp. 183—235.
- Dekhuijzen, H. M., Van Der Scheer, C. (1969): The ultrastructure of powdery mildew Sphaerotheca fuliginea, isolated from cucumber leaves. Neth. J. Plant Pathol. **75**: 169—177.
- Ebrahim-Nesbat, F., Heitefuss, R., Rohringer, R. (1986): Ultrastructural and histochemical studies on mildew of barley (Erysiphe graminis DC. f.sp. hordei Marchal). IV. Characterisation of papillae in fifth leaves exhibiting adult plant resistance. Phytopathol. Z. **177**: 289—300.
- Edwards, M. C., Allen, P. J. (1970): A fine structure study of the penetration process during infection of barley by Erysiphe graminis DC. f.sp. hordei. Phytopathol. **60**: 1504—1509.
- Edwards, M. C., Ayres, P. G. (1981): Cell death and cell wall papillae in the resistance of oak species to powdery mildew disease. New Phytol. **89**: 411—418.
- Ehrlich, H. G., Ehrlich, M. A. (1963): Electron microscopy of the host-parasite relationship in stem rust of wheat. Amer. J. Bot. **50**: 123—130.
- Ehrlich, M. A., Ehrlich, H. G. (1971): Fine structure of the host-parasite interfaces in myco-parasitism. Ann. Rev. Phytopathol. **9**: 155—184.
- Esashi, Y., Leopold, A. C. (1969): Cotyledon expansion as a bioassay for cytokinin. Plant Physiol. **44**: 618—620.
- Foda, H. A., Radwan, S. S. (1962): Straight growth test for hormones and inhibitors using coleoptile of some Egyptian gramineous plant. Ain Shams, Sci. Bull. **8**: 381—399.
- Frankland, B., Wareing, P. F. (1960): Effect of gibberellic acid on hypocotyl growth of lettuce seedling. Nature **23**: 255—265.
- Gil, F., Gay, J. L. (1977): Ultrastructural and physiological properties of the host interfacial components of haustoria of Erysiphe pisi in vivo and vitro. Physiol. Plant. Pathol. **10**: 1—12.
- Gorter, G. J. M. A. (1966): Powdery mildew fungus on cucurbits in the Transvaal province of South Africa. Nature **204**: 938—939.
- Heath, M. C., Stumpf, M. A. (1986): Ultrastructural observation of penetration sites of the cowpea rust fungus in untreated and silicon depleted French bean cells. Physiol. Plant Pathol. **29**: 27—39.
- Hewitt, H. G., Ayres, P. G. (1976): Effect of infection by Microsphaera alphitoides (powdery mildew) on carbohydrate levels and translocation in seedling of Quercus robur. New Phytol. **77**: 379—390.
- Hirata, K. (1937): The haustoria of some powdery mildews. Ann. Phytopathol. Soc. Japan **6**: 319—334.
- Hirata, K., Kojima, M. (1962): On the structure and the sack of the haustorium of some powdery mildews, with some consideration on the significance of the sack. Trans. Mycol. Soc. Japan **3**: 43—46.
- Hwang, B. K., Heitefuss, R. (1986): Sugar composition and invertase activity in spring barley plants in relation to adult plant resistance to powdery mildew. Phytopathol. **76**: 365—369.
- Ibrahim, I. F., Kadhim, L. J., Mahmood, A. H. (1985): A study of some biological characters for Sphaerotheca fuliginea as new powdery mildew causative agent on cucumber in Iraq. J. Biol. Sci. Res. **16**: 1—12.
- Jensen, W. A. (1962): Botanical Histochemistry. W. H. Freeman et Co. San Francisco and London.

- Jindal, S., Arora, Y. K., Bajaj, L. (1979): Effect of infection of Sphaerotheca fuliginea (powdery mildew) on amino acids sugars in leaves of muskmelon (Cucumis melo L.). The Plant Biochem. J. 6: 115-123.
- Kabasch, U. (1982): Changes in carbohydrate metabolism in cucumber leaves infected by Sphaerotheca fuliginea. Zeitschrift J. Pflanzenkrankheiten und Pflanzenschutz, 89: 113-124.
- Kenigsbuch, D., Cohen, Y. (1989): Independent inheritance of resistance to race 1 and race 2 of Sphaerotheca fuliginea in muskmelon. Plant Disease, 73: 206-208.
- Kojima, M., Hirata, K. (1961): On the sack of the haustorium of barley powdery mildew. (Abstr.) Ann. Phytopathol. Soc. Japan, 26: 60.
- Kosuge, T. (1978): The capture and use of energy by disease plants. In: Horsfall, J., Cowling, E. B. (eds): Plant Disease. VIII. How plant suffer from disease. Acad. Press, London.
- Kunoh, H., Kohno, M., Tashiro, S., Ishizaki, H. (1979): Studies of powdery mildew fungus, Leveillula taurica, on green pepper. II. Light and electron microscopic observation of the infection process. Can. J. Bot. 57: 2501-2508.
- Latham, D. B., Priest, M. J. (1989): Occurrence of cleistothecia of Sphaerotheca fuliginea, on cucurbits in South Australia and New South Wales. Austral. Plant Pathology, 18: 35-37.
- Manners, J. M., Gay, J. L. (1982): Transport, translocation and metabolism of ¹⁴C-photosynthates at the host-parasite interface of Pisum sativum and Erysiphe pisi. New Phytol. 91: 221-224.
- McKeen, W. E., Smith, R., Mitchell, N. (1966): The haustorium of Erysiphe cichoracearum and the host-parasite interface on Helianthus annuus. Can. J. Bot. 44: 1299-1306.
- Metzner, H., Rau, H., Singer, H. (1965): Untersuchungen zur Synchronisierbarkeit einzelner Pigment-Mangel Mutanten von Chlorella. Planta (Berlin) 65: 186-194.
- Mirocha, C. J., Zaki, A. I. (1966): Fluctuation in the amount of starch in host plants invaded by rust and mildew fungi. Phytopathol. 56: 1220-1224.
- Mundy, J. (1984): Hormonal regulation of amylase inhibitor synthesis in germinating barley. Carlsberg Res. Communic. 49: 439-444.
- Perera, R., Gay, J. L. (1976): The ultrastructure of Sphaerotheca pannosa (Wallroth ex Fries) Leveille and associated cells of rose. Physiol. Plant Pathol. 9: 57-65.
- Peyton, G. A., Bowen, C. C. (1963): The host-parasite interface of Peronospora manshurica on Glycine max. Am. J. Bot. 50: 787-797.
- Pratt, C., Gaffinet, M. C., Welser, M. J., Pearson, R. C. (1984): Powdery mildew of Vitis: papillae (wall apposition) as a host response to infections. Vitis 23: 225-229.
- Reifschneider, F. J. B., Boiteux, L. S., Occhiena, E. M. (1985): Powdery mildew of melon, Cucumis melo caused by Sphaerotheca fuliginea in Brazil. Plant Disease 69: 1069-1070.
- Reynolds, E. S. (1963): The use of lead citrate at a high pH as an electron opaque strain in electron microscopy. J. Cell Biology 17: 208-212.
- Roberts, D. A., Boothroyd, C. W. (1989): Fundamental of plant pathology. 2nd ed. 333 pp. W. H. Freeman and Co., N. Y.
- Schipper, A. L., Mirocha, C. J. (1969): The histochemistry of starch depletion and accumulation in bean leaves at rust-infection sites. Phytopathol. 59: 1416-1422.
- Shaw, M., Manocha, M. S. (1965): The physiology of host-parasite relation. XV. Fine structure in rust-infected wheat leaves. Can. J. Bot. 43: 1285-1292.
- Shindy, W. W., Smith, D. E. (1975): Identification of plant hormones from cotton ovules. Plant Physiol. 55: 550-554.

- Siradhana, B., Ghandhari, S. S. (1972): Occurrence of Erysiphe cichoracearum and Sphaerotheca fuliginea of Udaipar, Rajasthan (India). Indian J. Mycol. Plant Path. 1: 76-79.
- Smart, M. J., Aist, J. R., Israel, H. W. (1986a): Structure and function of wall appositions. I. General histochemistry of papillae in barley coleoptiles attacked by Erysiphe graminis f.sp. hordei. Can. J. Bot. 64: 793-801.
- Smart, M. J., Aist, J. R., Israel, H. W. (1986b): Structure and function of wall appositions. II. Callose and the resistance of oversize papillae to penetration by Erysiphe graminis f.sp. hordei. Can. J. Bot. 64: 802-804.
- Smith, D., Muscatine, L., Lewis, D. H. (1969): Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. Biol. Rev. 44: 17-90.
- Sokolov, Yu. V. (1978): Identification of the pathogen of powdery mildew on Cucurbitaceae in the Asrakhan region. Mikologiya 3: 264-266.
- Stoddart, J. L. (1981): Leaf senescence. In: Jeffcoat, B. (ed.): Aspects and Prospects of Plant Growth Regulators, pp. 51-63. British Plant Growth Regulators Group. ARC Letcombe Labor. Wantage, Oxfordshire.
- Takaki, M., Dietrich, M. C. (1980): Effect of Ga_3 and light on polysaccharides levels and metabolism in germinating coffee seeds. Exp. Bot. 31: 164-167.
- Whipps, J. M., Cooke, R. C. (1977): Comparative physiology of Albugo tragopogonis and Puccinia lagenophorae infected plants of Senecio squalidus L. New Phytol. 81: 307-319.
- Woods, A. M., Gay, J. L. (1987): The interface between haustoria of Puccinia poarum (monokaryon) and Tussilago farfara. Physiol. Plant. Pathol. 30: 167-185.
- Wright, S. T. C. (1956): Studies on fruit development in relation to plant hormones. III. Auxins in relation to fruit morphogenesis and fruit drop in the black-current (Ribes nigrum). J. Hort. Sci. 31: 196-211.
- Yarwood, C. E. (1978): History and taxonomy of powdery mildews. In: Spencer, D. M. (ed.): The Powdery Mildews, pp. 1-37. Acad. Press, London, N. Y., San Francisco.
- Younis, A. E., Younis, M. E., Gaber, M. A. (1969): Studies on the effect of certain enzymic poisons on metabolic of storage organs. II. Differential effects of iodoacetate on the respiration metabolism and permeability barriers in radish root slices. Plant and Cell Physiol. 10: 95-101.

Plate I. SE micrographs

Fig. 1. The hyphae are spreading on the surface of squash leaf, the hyphae giving rise conidiophores with conidia. Bar: 50 μ m

Fig. 2. Single conidiophore (cd) with unseparated conidium (cn). Note separated conidium. Bar: 5 μ m

Fig. 3. Single conidium (cn) with wrinkled surface. Bar: 5 μ m

Plate II. LM micrographs

Fig. 4. Numerous hyphae (h) are located on the host epidermis (E). Most of the epidermal cells are invaded by one or two haustoria (H). Note the penetration peg (arrow). Note also the chloroplasts (arrowheads) of mesophyll tissue which contain several starch grains. Resin-embedded section. Bar: 10 μ m

Fig. 5. Two multilayered cleistothecia. Wax-embedded section. Bar: 10 μ m

Fig. 6. A cleistothecium contains one ascus (A) with ascospores (As). Wax-embedded section. Bar: 5 μ m

Fig. 7. A cleistothecium with one ascus (A) and ascospores (As). Note appendages (arrows) emerging from the multilayered wall. Wax-embedded section. Bar: 5 μ m

Plate III. TE micrographs

Fig. 8. Host epidermis (E) is penetrated by a penetration peg (pg) which is enclosed by a collar (CO). Numerous vesicles (arrowheads) are observed inside the collar. The host plasma-lemma (large arrows) is invaginated around the collar. Note an electron-lucent area (small arrows) on both sides of the peg. Note also host mitochondria (M) and tonoplast (T). Bar: 1 μ m

Fig. 9. A haustorium (H) with single nucleus (n) and small vacuoles (v). The haustorium is surrounded by a wide matrix (Mt) which contains several haustorial lobes (L). Note the invaginated host plasmalemma (arrows). Bar: 3 μ m

Plate IV. TE micrographs

Fig. 10. A chloroplast from healthy leaf showing well developed membrane system. Note few plastoglobuli (arrows) and single starch grain (s). Bar: 1 μ m

Fig. 11. Three chloroplasts from infected leaf showing disorganisation of granal system and intergranal lamellae. Note the increase in number of starch grains (s) and plastoglobuli (arrows) than Fig. 10. Bar: 2 μ m

Plate I

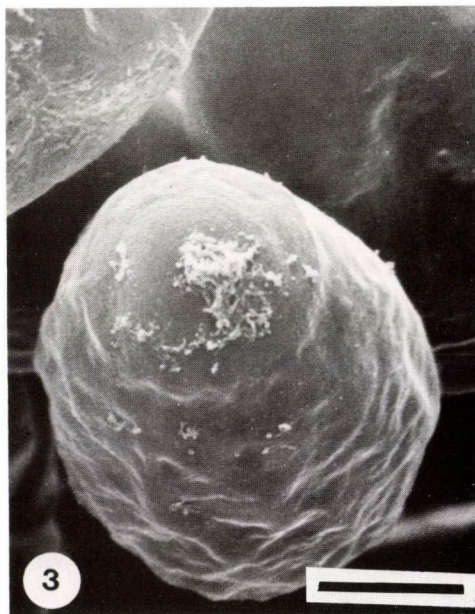
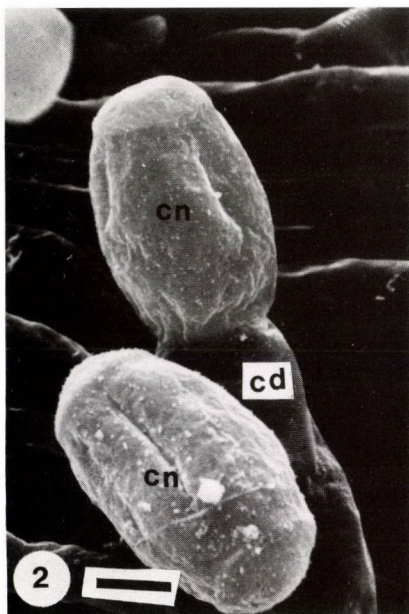


Plate II

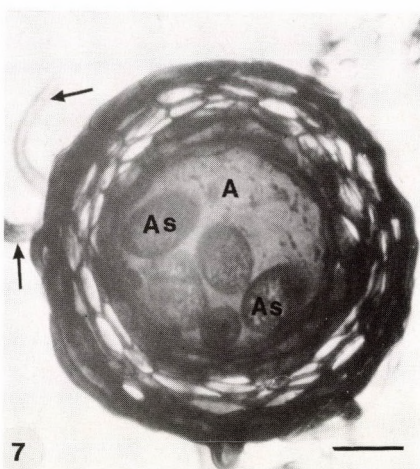
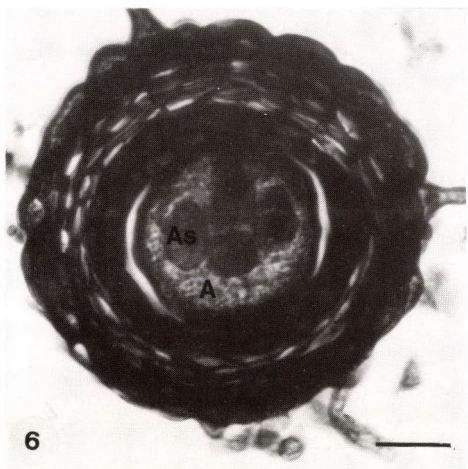
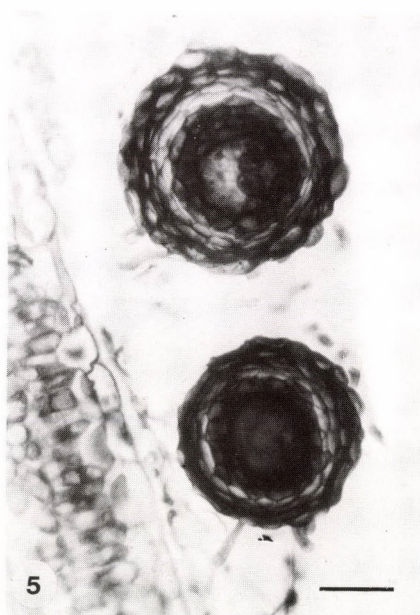
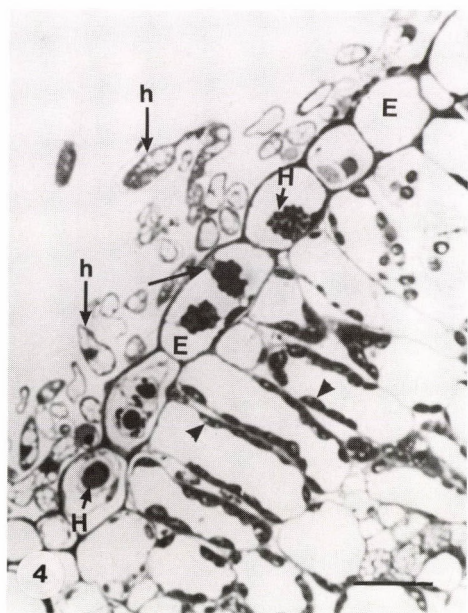


Plate III

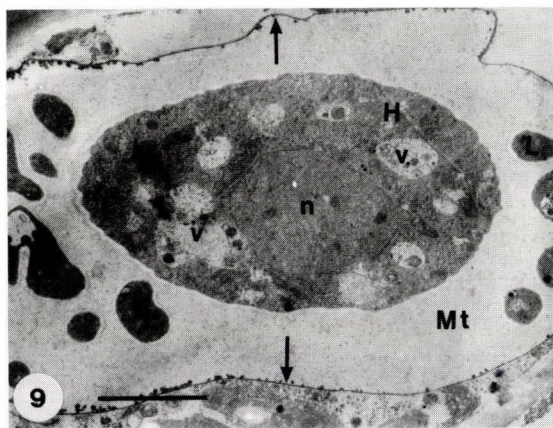
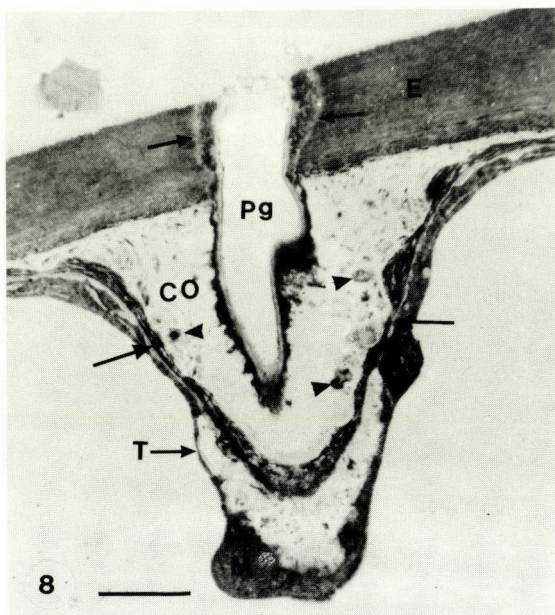
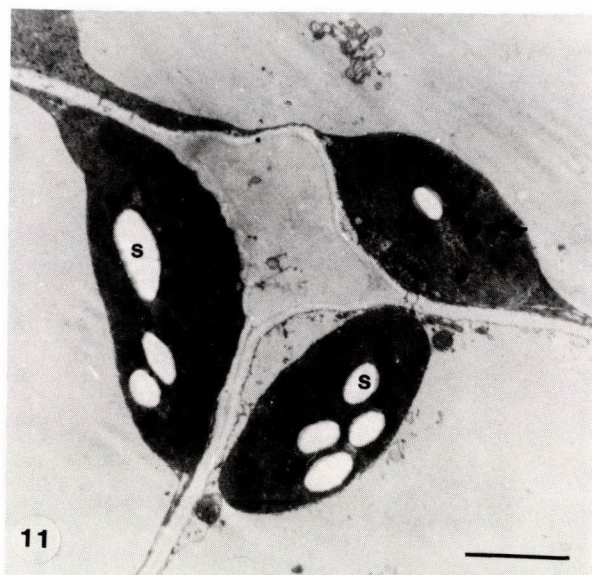
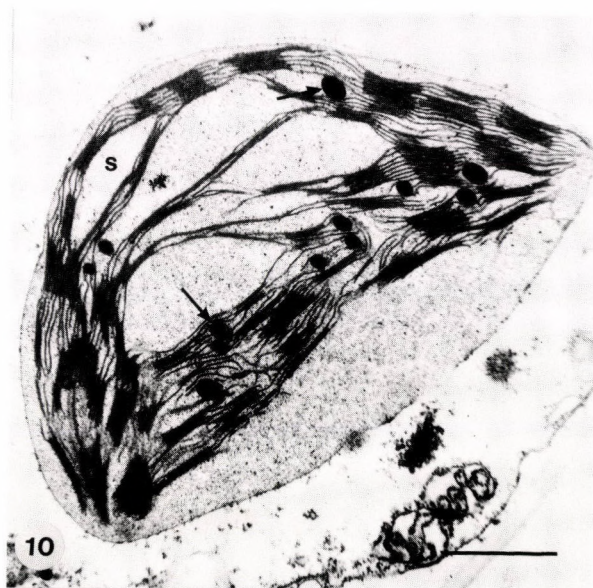


Plate IV



EL GÉNERO *ALTERNARIA* (HYPHOMYCETES, DEUTEROMYCOTINA) EN CUBA

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A taxonomic study of species from genus *Alternaria* Nees ex Fries founded in Cuba is pointed out. This genus is of a great practical interest because mostly of its species are pathogens more or less severe of different cultures of economic importance. We find 18 species which are described and illustrated. We offer for the first time in Cuba a dichotomic key of such *Alternaria* species.

Introducción

El presente trabajo tiene como objetivo la continuación de los estudios taxonómicos que sobre los hifomicetes de Cuba se vienen llevando a cabo dentro del problema Fundamental Flora de la República de Cuba.

En este caso el género que presentamos es de un gran interés práctico, sobre todo para la agricultura, pues la mayoría de las especies de que consta están consideradas como patógenas más o menos severas de diferentes cultivos de importancia económica.

Parte de las especies descritas pertenecen a las colectas personales de los autores de este trabajo y fueron convenientemente determinadas por los mismos. No obstante, se han añadido también algunas especies no halladas por nosotros, pero reportadas para Cuba por otros investigadores y cuyo material se encuentra, en muchos casos, depositado en otras instituciones cubanas o extranjeras. Varias de esas especies pudieron ser revisadas y hemos considerado útil el incluirlas aquí.

En total se describen e ilustran 18 especies de *Alternaria*. Se confeccionó también, por primera vez para Cuba, una clave dicotómica de las especies descritas, lo que facilitará la correcta identificación de las mismas. Dicha clave se elaboró tomando en cuenta como caracteres principales la formación de conidios solitarios o en cadenas, la presencia o no de cuello y su longitud, así como la especificidad por determinados hospederos.

Reseña taxonomica

Alternaria Nees ex Fr.

Nees, Syst. Pilze Schwamme, p. 72, 1816; Fries, Syst. Mycol. 1: XLVI, 1821.

Syn.: Macrosporium Fr., Syst. Mycol. 3: 373. 1832. Rhopalidium Mont., Ann. Sci. Nat. Ser. 2. 6: 30, 1846.

Especie tipo: Alternaria alternata (Fr.) Keissler.

Colonias dispersas, usualmente grises, pardo negruzcas o negras, aterciopeladas o velutinosas. Micelio inmerso o en parte superficial, compuesto por hifas subhialinas, pardo oliváceas o pardas. Estroma casi nunca presente. Sin setas ni hifopodios conidióforos macronemáticos, mononemáticos, sin ramificar o poco e irregularmente ramificados, solitarios o en fascículos, pardo pálidos o pardos. Células conidiógenas politréticas, integradas, terminales, más tarde intercaladas, simpodiales, a veces monotréticas, cicatrizadas. Conidios catenulados o solitarios, secos, obovoides u obclaviformes, raramente cilíndricos, a menudo rostrados, con septos transversales y también longitudinales y, a veces, oblicuos, con un cuello largo o corto, a veces sin cuello, pardo pálidos, pardo oliváceos o pardos, lisos o verrugosos.

Las especies de este género son mayormente cosmopolitas y comunes, abundando especialmente en los trópicos, sobre un amplio rango de hospederos. Son mayormente parásitas de cultivos importantes o saprofitas sobre hojas y tallos muertos. Las patógenas constituyen importantes hongos que causan manchas foliares, pudriciones en raíces y frutos en numerosas plantas de interés agrícola, así como la marchitez de las posturas.

Los teleomorfos de las especies de Alternaria pertenecen mayormente al género Pleospora Rabenh. ex Ces. et de Not., Comm. Soc. Critt. Ital. 1: 217. 1863.

Clave para las especies de Alternaria halladas en Cuba.

Clave para las secciones

- I. Conidios en largas cadenas hasta de 10 o más, sin cuello o con cuello relativamente corto Sección Longicatenatae
- II. Conidios en cortas cadenas, usualmente de 3-5, con cuello muy corto o algo largos Sección Brevicatenatae
- III. Conidios solitarios, a veces se forma un conidio secundario, con cuello largo más o menos filiforme, raramente con cuello corto Sección Noncatenatae

Clave para las especiesI. Longicatenatae

- 1 a) Conidios obclaviformes, ovoides o elipsoidales con cuello cilíndrico, hasta con 8 septos transversales y algunos septos longitudinales, de color pardo dorado pálido a pardo dorado; cosmopolita o patógeno ocasional A. alternata
- 1 b) Conidios cilíndricos u obclaviformes, casi siempre sin cuello, aproximadamente con 6 septos transversales y algunos longitudinales, de color pardo oliváceo pálido a pardo oliváceo oscuro; patógeno sobre Cruciferae A. brassicicola

II. Brevicatenatae

- 1 a) Conidios con cuello relativamente corto en relación al cuerpo de la espora 2
- b) Conidios con cuello más o menos largo en relación al cuerpo de la espora 4
- 2 a) Conidios de no más de 60 μm de largo (incluyendo el cuello); patógeno sobre Citrus A. citri
- b) Conidios usualmente de más de 60 μm de largo 3
- 3 a) Conidios de 50-130 x 14-30 μm , fuertemente constreñidos en los septos; patógeno sobre Raphanus A. raphani
- b) Conidios de 55-125 x 14-24.5 μm , ligeramente constreñidos en los septos; patógeno sobre Sonchus y Lactuca A. sonchi
- 4 a) Conidios usualmente con más de 20 μm de grueso en la parte más ancha, de 65-320 x 19-32 μm ; patógeno sobre Cruciferae A. brassicae
- b) Conidios usualmente con menos de 20 μm de grueso en la parte más ancha; saprófitos o patógenos de otras plantas pero no de Cruciferae 5
- 5 a) Conidios de 35-110 x 11-21 μm ; patógeno sobre Nicotiana A. longipes
- b) Conidios de 20-85 x 7-18.5 μm , saprófito cosmopolita o patógeno débil sobre diversas plantas A. tenuissima

III. Noncatenatae

- 1 a) Conidios sin cuello o con cuello relativamente corto en relación al cuerpo de la espora 2
- b) Conidios con cuello largo, usualmente filiforme 3
- 2 a) Conidios mayormente cilíndricos, redondeados en los extremos, sin cuello, de 35-140 x 11-30 μm ; patógeno sobre Helianthus ... A. helianthi

- b) Conidios obclaviformes y rostrados, con cuello hasta de un tercio de la longitud de la espora, de 70-140 x 11-15 μm ; patógeno sobre Gomphrena A. gomphrenae
- 3 a) Conidios con el cuello a menudo ramificado 4
- b) Conidios con el cuello casi siempre sin ramificar 5
- 4 a) Conidios con el cuello hasta 3 veces la longitud del cuerpo de la espora, de 100-450 x 16-25 μm ; patógeno sobre Daucus A. dauci
- b) Conidios con el cuello de longitud igual o algo más largos que el cuerpo de la espora, de 90-260 x 8-16 μm ; patógeno sobre Solanaceae A. solani
- 5 a) Conidios variables en su forma, mayormente muy largos, de 35-220 x 7-10 μm , presencia de clamidosporas; patógeno débil o saprófito A. longissima
- b) Conidios no variables en su forma; sin formación de clamidosporas; patógenos de diversas plantas 6
- 6 a) Patógeno sobre Ricinus; cuello igual o dos veces mayor que el cuerpo de la espora; conidios de 70-170 x 13-27 μm A. ricini
- b) Patógeno sobre Gossypium; cuello igual o dos veces mayor que el cuerpo de la espora; conidios de 90-180 x 15-22 μm A. macrospora
- c) Patógeno sobre Allium; cuello a menudo igual en longitud al cuerpo de la espora, pero puede ser mayor o menor a éste; conidios de 90-270 x 12-18.5 μm A. porri
- d) Patógeno sobre Cucurbitaceae; cuello más largo que el cuerpo de la espora; conidios de 110-200 x 12-22 μm A. cucumerina
- e) Patógeno sobre Datura; cuello más largo que el cuerpo de la espora; conidios de 120-440 x 15-40 μm A. crassa

Alternaria alternata (Fr.) Keissler (Fig. 1/1)

Beih. Bot. Zbl. 29: 434: 1912.

Syn.: Torula alternata Fr., Syst. mycol. 3: 500. 1832.

Alternaria tenuis C.G. Nees, Syst. Pilze Schwamme, p. 72. 1816/17 (nombre no válido).

Colonias dispersas, usualmente negras o negro oliváceas, a veces grises. Micelio parte inmerso en el sustrato. Conidióforos solitarios o en pequeños grupos, sin ramificar o ramificados, rectos o flexuosos, a veces geniculados, septados, pardo pálidos, pardo oliváceos o pardo dorados, lisos, hasta de 50 μm de largo por 2.5-5.5 μm de grueso. Conidios en cadenas largas y a menudo ramificadas, obclaviformes, obpiriformes, ovoides o elip-

soidales, hasta con 8 septos transversales y varios septos longitudinales y oblicuos, de color pardo pálido a pardo dorado, de 18-65 μm de largo por 8-17.5 μm de grueso en la parte más ancha, a menudo con un cuello corto, cónico o cilíndrico, a veces hasta de un tercio de la longitud del conidio, pero no más, pálido, de 2.2-4.5 μm de grueso.

Hábitat: un saprófito en extremo común, reportado sobre muy diversas plantas como Barringtonia speciosa Will., Bougainvillea spectabilis Willd., Brassia actinophylla F.Muell., Capsicum annum L., Crotalaria latifolia L., Gossypium hirsutum L., Leptilon pusillum (Nutt.)Britt., Lycopersicum esculentum L., Nicotiana tabacum L., y sobre palmácea no determinada. También se ha hallado sobre otros sustratos tales como alimentos, suelo y textiles.

Material colectado en Cuba: Prov. Ciudad de la Habana, Jardín Botánico de la Habana, col. A. MERCADO, 29. XI. 1986. (HACM 8392).

Distribución: cosmopolita.

Alternaria brassicae (Berk.) Sacc. (Fig. 2)

Michelia, 2: 129. 1880.

Syn.: Macrosporium brassicae Berk., en Smith's Engl. Fl. 5. pt. 2: 339. 1836.

Colonias dispersas, pardo oliváceas, pelosas, anfígenas. Micelio inmerso compuesto por hifas ramificadas, hialinas, septadas, lisas, de 3-7 μm de grueso. Conidióforos que surgen en grupos de 2-10 o más, de las hifas del micelio, emergiendo a través de los estomas, usualmente sin ramificar, erectos o ascendentes, rectos o flexuosos, frecuentemente geniculados, más o menos cilíndricos, pero a menudo ligeramente abultados en la base, oliváceo grisáceo pálidos, septados, lisos, hasta de 150 μm de longitud por 5.5-10 μm de grueso, con una o varias cicatrices, con poros conidiales pequeños pero discernibles. Conidios solitarios u ocasionalmente en cadenas hasta de 4, rectos o ligeramente curvos, obclaviformes, rostrados, oliváceo pálidos o gris oliváceos, con 7-17 septos transversales y 0-7 septos longitudinales u oblicuos, lisos, infrecuentemente con verrugas muy poco notables, de 65-320 μm de largo por 19-32 μm de grueso en la parte más ancha, con el cuello de un tercio a un medio de la longitud del conidio y de 5-9 μm de grueso.

Habitat: Parásito sobre hojas de Brassica napus L., de Brassica oleracea var. capitata L., de Brassica rapa L., de Brassica sp., y de Crambe abbyssinica Hochst.

Material colectado en Cuba: Prov. Habana, Catalina de Guines (KREISEL 1971). Para lista de hospederos consultar a FERNÁNDEZ ROSEÑADA (1973), SEIDEL (1976) y ARNOLD (1986).

Distribución: cosmopolita.

Sobre hojas de varias crucíferas forma manchas circulares zonadas, ligeramente pardas a grisáceas u oscuras, de 0.5-12 mm de diámetro, a veces coalescentes. Sobre las nervaduras medias de las hojas, las manchas son oblongas o lineales, hundidas, y sobre cabezas de coliflor se forman manchas negras. Entre las plantas hospedadoras se incluyen el brocolí, la col, colirrábano, mostaza, nabo, rábano y rabanillo (según ELLIS 1971).

Alternaria brassicicola (Sch.) Wiltshire (Fig. 1/2)

Mycol. Pap. 20: 8. 1947.

Syn.: Helminthosporium brassicicola Schweinitz, Trans. Am. Phil. Soc., N. Ser. 4: 279. 1832.

Macrosporium cheiranthi Fr. var. circinans Berk et Curt. Grevillea, 3: 105. 1875.

Alternaria oleracea Milbraith, Bot. Gaz. 74: 320. 1922.

Alternaria circinans (Berk. et Curt.) Bolle, Meded. Phytopath. Lab. Willie Commelin Scholten 7: 26. 1924.

Colonias dispersas, pardo oliváceo oscuras a pardo negruzcas, anfígenas, aterciopeladas. Micelio inmerso en el sustrato compuesto por hifas ramificadas, hialinas al principio, más tarde pardas a pardo oliváceas, intercelulares e intracelulares, septadas, lisas, de 1.5-7.5 μ m de grueso. Conidióforos solitarios o en grupos de 10-12 o más, que emergen a través de los estomas, usualmente sin ramificar, erectos o ascendentes, rectos o curvos, ocasionalmente geniculados, más o menos cilíndricos, pero a menudo ligeramente abultados en la base, pardo pálidos a pardo oliváceos, septados, lisos, hasta de 70 μ m de largo por 5-8 μ m de grueso. Conidios mayormente en cadenas hasta de 20 o más, a veces ramificados, que surgen a través de pequeños poros en la pared del conidióforo, rectos, casi cilíndricos, usualmente aguzándose ligeramente hacia el ápice, u obclaviformes, con la célula basal redondeada, casi siempre sin cuello, la célula apical más o menos rectangular o semejando un cono truncado, de 6-8 μ m de grueso, de color pardo oliváceo pálido a pardo oliváceo oscuro, con 1-11 (mayormente con menos de 6) septos transversales y usualmente 0-6 septos longitudinales y oblicuos, a menudo constreñidos en los septos, lisos o ligeramente verrugosos con la edad; de 18-130 μ m de longitud por 8-20 μ m de grueso en la parte más ancha.

Habitat: Sobre Brassica oleracea var. capitata L.

Material colectado en Cuba: Prov. de Holguín, Limones, 1966. (IMI 117215). Ver URTIAGA (inédito), SEIDEL (1976) y ARNOLD (1986).

Distribución: Cosmopolita, común en Australia, Birmania, Canadá, Chipre, Cuba, EE.UU., Etiopía, Europa (parte), Ghana, Guinea, Hong Kong, India, Inglaterra, Jamaica, Japón, Libia, Nigeria, Rumania, Sabah, Sierra Leona, Sudáfrica, Sudán, Sri Lanka, Taiwan, Tanzania, Turquía, Uganda, Zambia, Zimbabwe.

Este hongo es un serio parásito sobre hojas de diferentes crucíferas a las cuales causa manchas zonales y circulares de color pardo oscuro a negro.

Es más común y causa una enfermedad más severa que Alternaria brassicae en semilleros. La col blanca y la coliflor son objeto de fuertes ataques. La berza es relativamente inmune. Puede causar considerable daño a la col y a la coliflor en transito (según ELLIS 1971).

Alternaria citri Ellis et Pierce apud Pierce (Fig. 3/1)

Bot. Gaz. 33: 234. 1902.

Colonias dispersas, oliváceas a negras, en cultivo son grises, pardo oliváceas a negras. Micelio parte inmerso en el sustrato. Conidióforos sin ramificar o ramificados, rectos o flexuosos, pardo pálidos a pardo oliváceos, septados, lisos, con una cicatriz terminal y a veces una o dos laterales, hasta de 300 μm de largo por 3-5 μm de grueso. Conidios solitarios o en cadenas ramificadas de 2-7, rectos o ligeramente curvos, de forma variada, pero comúnmente obclaviformes u ovoides, a menudo rostrados, pardo pálidos, pardo oliváceos o a veces pardo oscuros, hasta con 8 septos transversales y numerosos septos longitudinales u oblicuos, constreñidos en los septos, lisos o verrugosos, de 8-60 μm de largo incluyendo el cuello cuando está presente, por 6-24 μm de grueso en la parte más ancha; cuello mayormente de 8 μm o menos de longitud por 2.5-4 μm de grueso, hialino o pardo pálido.

Hábitat: Sobre hojas y frutos de Citrus sinensis Osbeck y de otras especies de cítricos.

Material colectado en Cuba: Ver FERNÁNDEZ ROSEÑADA (1973), SEIDEL (1976) y ARNOLD (1986).

Distribución: Argentina, Australia, Birmania, Bulgaria, China, Chipre, Cuba, EE.UU., Egipto, España, Francia, Grecia, India, Inglaterra, Irán, Israel, Italia, Jamaica, Japón, Kenya, Libia, Malawi, Malta, Portugal, Puerto Rico, Sudáfrica, Sudán, Tanzania, Uganda, Uruguay, URSS, Zambia, Zimbabwe.

Responsable de varios tipos de daños a los frutos y hojas de Citrus spp. como son la pudrición negra de las naranjas y la pudrición de los limoneros.

Alternaria crassa (Sacc.) Rands (Fig. 5)

Phytopathology 7: 327--338. 1917.

Syn.: Cercospora crassa Sacc. Michelia 1: 88. 1877.

Cercospora daturae Peck, Rep. N. Y. St. Mus. Nat. Hist., 35: 140. 1882.

Macrosporium daturae Fautray, Revue mycol. 16: 76. 1894.

Alternaria daturae (Fautrey) Bubák et Ranojevic, Fungi Imperfecti Exsicc. No. 694. 1909.

Colonias dispersas. Micelio mayormente inmerso en el sustrato. Conidióforos solitarios o en pequeños grupos, erectos o ascendentes, rectos o flexuosos, a veces geniculados, pardo pálidos, septados, lisos, hasta de 90 μ m de largo por 7-10 μ m de grueso, con una o varias cicatrices. Conidios usualmente solitarios, ocasionalmente en cadenas muy cortas, obclaviformes, rostrados, pardo pálidos, con 7-10 septos transversales y varios septos longitudinales u oblicuos, a menudo constreñidos en los septos, lisos, de 120-440 μ m de largo (cuerpo hasta de 140 μ m de largo), por 15-40 μ m de grueso en la parte más ancha; cuello pardo pálido, septado, sin ramificar, mucho más largo que el cuerpo, de 4-8 μ m de grueso en la base, adelgazando hasta 2-2.5 μ m.

Hábitat: Sobre hojas de Datura stramonium L.

Material colectado en Cuba: En el presente este hongo no se ha colectado en Cuba, pero anteriormente fue reportado por ELLIS (1971).

Distribución: Alemania, Chipre, Cuba, España, EE.UU., Etiopía, Ghana, India, Italia, Kenya, Nepal, Nigeria, Paquistán, Rumania, Sudán, Suiza, Tanzania, Uganda, Zambia, Zimbabwe.

Causa manchas irregulares, zonales, de color pajizo, las cuales aparecen primero sobre las hojas más bajas y umbrías, esparciéndose gradualmente hacia arriba.

Alternaria cucumerina (Ellis et Everh.) Elliot (Fig. 4/2)

Am. J. Bot. 4: 472. 1917.

Syn.: Macrosporium cucumerinum Ellis et Everh., Proc. Acad. Nat. Sci. Philad. 1895: 440, 1895.

Colonias dispersas, anfígenas. Micelio mayormente inmerso en el sustrato. Conidióforos solitarios o en pequeños grupos, erectos, rectos o flexuosos, a

veces geniculados, cilíndricos, pardo pálidos a pardos, septados, lisos, hasta de 100 μm de largo por 5-9 μm de grueso, usualmente con varias cicatrices conidiales. Conidios solitarios u ocasionalmente en cadenas de 2, obclaviformes, rostrados, con el cuello muy largo, pardo pálidos a pardo dorados, con 6-9 septos transversales y a veces muchos septos longitudinales y oblicuos, lisos o verrugosos, de 110-200 μm de largo por 12-22 μm de grueso en la parte más ancha; cuello pardo pálido, septado, sin ramificar, a menudo mucho más largo que el cuerpo, de 3.5-5 μm de grueso en la base, estrechándose hasta 1-2.5 μm .

Hábitat: Patógeno sobre hojas de Cucumis sativus L., de Cucumis melo y de Cucurbita pepo L.

Material colectado en Cuba: Prov. Granma, Peralejo, Bayamo, 1966; La Pupa, Bayamo, 1966. Prov. de Guantánamo, Mata Abajo, 1966 (URTIAGA inédito). Ver también SEIDEL (1976) y ARNOLD (1986).

Distribución: Arabia Saudita, Australia, Canada, Chile, Chipre, Cuba, EE.UU., Francia, Kenya, Libia, Nueva Zelandia, Nigeria, Sierra Leona, Sudáfrica, Sudán, Trinidad, Venezuela, Zambia, Zimbabwe.

Causa el tizón de la hoja de las cucurbitáceas, a menudo de importancia económica. Las manchas son primero pequeñas, circulares, blanquecinas o de color canela, más tarde se expanden y se hacen a menudo zonales, con un margen pardo claro sobre el lado superior de la hoja. También aparece sobre los frutos y se dice que invierna en el suelo. Entre las plantas hospedadoras se incluyen la calabaza, el melón de agua, el de castilla y el pepino (según ELLIS 1971).

Alternaria dauci (Kühn) Groves et Skolko (Fig. 6)

Can. J. Res., Sect. C. 22: 222. 1944.

Syn.: Sporidesmium exitiosum Kuhn var. dauci Kuhn, Hedwigia, 1: 91. 1855.

Macrosporium carotae Ellis et Langlois, J. Mycol. 6: 36. 1890.

Alternaria carotae (Ellis et Langlois) Stevenson et Wellmann, J. Wash. Acad. Sci. 34: 263. 1944.

Colonias dispersas. Micelio mayormente inmerso en el sustrato. Conidióforos solitarios o en pequeños grupos, rectos o flexuosos, a veces geniculados, pardo oliváceo pálidos o pardos, septados, lisos, hasta de 80 μm de largo por 6-10 μm de grueso. Conidios usualmente solitarios, ocasionalmente en cadenas de 2, rectos o curvos, obclaviformes, rostrados, al principio pardo oliváceo pálidos, tornándose pardos con la edad, con 7-11 septos transver-

sales y uno o varios septos longitudinales y oblicuos, de 100-450 μm de largo por 16-25 μm de grueso en la parte más ancha; cuello a menudo ramificado. alcanzando hasta 3 veces el largo del cuerpo del conidio, flexuoso, hialino o pálido, de 5-7 μm de grueso en la base, adelgazando hasta 1-3 μm .

Hábitat: Sobre hojas de Daucus carota L. var. sativa D.C.

Material colectado en Cuba: prov. Granma, Bayamo, 1966 (URTIAGA inédito). Ver también SEIDEL (1976) y ARNOLD (1986).

Distribución: Alemania, Australia, Austria, Bermudas, Brasil, Canada, Congo, Cuba, Dinamarca, EE.UU., Filipinas, Francia, Ghana, Honduras, India, Inglaterra, Israel, Jamaica, Japón, Kenya, Malawi, Malasia, Nicaragua, Perú, Puerto Rico, Sri Lanka, Sudáfrica, Salvador, Tanzania, Trinidad-Tobago, URSS, Venezuela, Zambia, Zimbabwe.

Causa el tizón de la hoja de la zanahoria y ha sido reportado sobre otras umbellíferas. Afecta las hojas y los pecioloos volviéndolos amarillos y luego pardos o negros.

Alternaria gomphrenae Togashi (Fig. 7/2)

Bull. Imp. Agric. For., Morioka 9: 1-16. 1926.

Colonias anfigenas, pero mayormente epífilas. Micelio inmerso en el sustrato. Conidióforos en fascículos, que emergen a través de los estomas, pardos, septados, lisos, hasta de 120 μm de largo por 6-9 μm de grueso, con 1-3 cicatrices. Conidios rectos o curvos, obclaviformes, rostrados, pardo dorados, con el cuello usualmente pálido y más corto que el cuerpo del conidio, mayormente hasta de un tercio de la longitud de la espora, con 8-11 septos transversales y raramente un septo longitudinal, de 70-140 μm de largo por 11-15 μm de grueso en la parte más ancha; cuello de 2-3 μm de grueso.

Habitat: Sobre hojas de Gomphrena globosa L.

Material colectado en Cuba: Reportado para Cuba por ELLIS (1976).

Distribución: Birmania, Cuba, India, Jamaica, Japón, Malasia, Sabah, Sri Lanka, Trinidad-Tobago.

Causa manchas hasta de 8 mm de diámetro, las cuales son orbiculares, de color amarillento a gris, con los bordes rojos o purpúreos (según ELLIS 1976).

Alternaria helianthi (Hansf.)Tubaki et Nishihara (Fig. 7/1)

Trans. Br. Mycol. Soc. 53: 147-149. 1969.

Syn.: Helminthosporium helianthi Hansford 1943.

Colonias dispersas, grises, finamente pelosas. Micelio parte inmerso en el sustrato. Conidióforos solitarios o en fascículos, sin ramificar, rectos o ligeramente flexuosos, a veces geniculados, pardi pálidos a pardo oliváceos, septados, lisos, hasta de 100 μm de largo por 6-9 μm de grueso. Conidios mayormente solitarios, pero ocasionalmente en cadenas de 2, rectos cilíndricos o raramente obclaviformes, redondeados en los extremos, subhialinos a pardo oliváceo pálidos o pardo dorados, sin cuello, con 2-10 septos transversales u ocasionalmente uno o más septos longitudinales y oblicuos, a veces constreñidos en los septos, lisos, de 35-140 μm de largo por 11-30 μm de grueso en la parte más ancha.

Hábitat: Sobre hojas, tallos y pétalos de girasol (Helianthus annuus L.).

Material colectado en Cuba: Prov. de Villa Clara, Empresa de Flores Sagua, 1984.

Distribución: Argentina, Australia, Cuba, India, Japón, Malawi, Tanzania, Uganda, Zambia.

Sobre hojas causa manchas pardas orbiculares las cuales son a veces zonadas o grises en el centro y pueden tener un margen amarillo; manchas negras, redondas o alargadas se forman sobre tallos y usualmente pequeñas manchas pardas sobre los pétalos (según ELLIS 1976).

Alternaria longipes (Ellis et Everh.) Mason (Fig. 8/1)

Mycol. Pap. 2: 19. 1920.

Syn.: Macrosporium longipes Ellis et Everh., J. Mycol. 7: 134. 1892. Colonias anfégenas. Micelio mayormente inmerso en el sustrato. Conidióforos solitarios o en grupos, erectos o ascendentes, sin ramificar o flojamente ramificados, rectos o flexuosos, cilíndricos, pardo oliváceo pálidos, septados, lisos, hasta de 80 μm de largo por 3-5 μm de grueso, con una o varias cicatrices conidiales. Conidios a veces solitarios, pero usualmente en cadenas, obclaviformes, rostrados, pálidos a pardo pálidos, con 5-6 septos transversales y uno a varios septos longitudinales u oblicuos, lisos o verrugosos; de 35-110 μm de longitud por 11-21 μm de grueso en la parte más ancha, adelgazando gradualmente hacia el cuello pardo pálido, que alcanza usualmente un tercio a un medio de la longitud total y mide 2-5 μm de grosor, abultándose ligeramente hacia el ápice.

Hábitat: Sobre hojas de Nicotiana tabacum L.

Material colectado en Cuba: Ver KREISEL (1971) y ARNOLD (1986).

Distribución: Alemania, Bolivia, China, Colombia, Congo, Cuba, EE.UU., Holanda, Hungría, India, Indonesia, Italia, Jamaica, Japón, Malawi, Marruecos, Mauritania, Mozambique, Nepal, Nueva Guinea, Panama, Paquistán, Polonia, Rumania, Sabah, Sudáfrica, Sudán, Tanzania, Uganda, Venezuela, Yugoslavia, Zambia, Zimbabwe.

Sobre tabaco causa la mancha parda. Las manchas aparecen primero en las hojas inferiores y son orbiculares, pardas y zonadas. Toda la hoja se vuelve parda y las manchas aparecen con un matiz más pálido que las áreas que la rodean. Las hojas superiores y a veces los tallos pueden infestarse (según ELLIS 1971).

Alternaria longissima Deighton et MacGarvie (Fig. 9)

Mycol. Pap. 113: 10. 1968.

Colonias dispersas, oscuras. Micelio parte superficial y parte inmerso en el sustrato. Conidióforos sin ramificar o flojamente ramificados, rectos o flexuosos, algo abultados en el ápice, pardo pálidos, septados, lisos, de 60-170 x 3.5-7 μm . Conidios solitarios o catenulados con diversidad en la forma y en el tamaño, a veces muy largos, obclaviformes o con un cuerpo basal subcilíndrico, compuesto por pocas células, con un cuello largo y estrecho, ocasionalmente con constricciones o nudosidades, mayormente con septos transversales y a veces con septos longitudinales y oblicuos, de 35-220 x 7-10 μm . Clamidosporas oscuras, multicelulares y muriformes aparecen a veces, con paredes más gruesas que los conidios, de 20-45 x 12-24 μm .

Hábitat: Sobre hojas de Annona squamosa L., de Digitaria insularis y sobre hojas de bromeliáceas.

Material colectado en Cuba: Prov. de Pinar del Río, Sierra del Rosario, Loma El Salón, col. A. MERCADO, 20. V. 1977 (HACM 2571; 1. IX. 1977 (HACM 2843). Prov. Granma, Bayamo, col. R. URTIAGA, 1967.

Distribución: Cuba, EE.UU., Egipto, Guinea, India, Lao, Malasia, Nepal, Nigeria, Sierra Leona, Sudán, Tanzania, Zambia.

También se ha reportado esta especie sobre granos de polen de maíz caídos, sobre cáscaras y granos de arroz y de Sorghum sp. y sobre hojas de muy diversas plantas, a menudo mezclada con otros hongos (ELLIS 1971).

Alternaria macrospora Zimm. (Fig. 10/2)

Ber. Land- u. Forstw. Dt. Ostaf. 2: 24. 1904.

Syn.: Sporidesmium longipedicillatum Reichert, Bot. Jb. 56: 723. 1921.

Alternaria longipedicellata Snowden, Uganda Rep. 1926: 31, 1927.

Colonias sobre hojas, anfigenas. Micelio parte inmerso en el sustrato. Conidióforos solitarios o en grupos, erectos, sin ramificar, rectos o flexuosos, casi cilíndricos o adelgazando ligeramente hacia el ápice, pardo pálidos a pardos, septados, lisos, con una o varias cicatrices conidiales, hasta de 80 μm de largo por 4-9 μm de grueso. Conidios solitarios o a veces en cadenas de 2, rectos o curvos, obclaviformes o con el cuerpo del conidio elipsoidal, adelgazando abruptamente hasta un cuello muy estrecho, pardo rojizos a pardo rojizo oscuros, con 4-9 septos transversales y varios septos longitudinales y oblicuos, a menudo ligeramente constreñidos en los septos, finamente verrugosos, de 90-180 μm de largo por 15-22 μm de grueso en la parte más ancha; cuello sin ramificar, pálido, que alcanza en longitud 1-2 veces la longitud del cuerpo, de 1-1.5 μm de grueso.

Hábitat: Sobre hojas de Gossypium hirsutum L. y de Gossypium barbadense L.

Material colectado en Cuba: Ver KREISEL (1971), FERNANDEZ ROSEÑADA (1973), SEIDEL (1976) y ARNOLD (1986).

Distribución: Australia, Brasil, China, Congo, Cuba, Etiopía, Francia, Ghana, Guadalupe, India, Italia, Japón, Malawi, Marruecos, Nigeria, República Centroafricana, Rumanía, Senegal, Sudáfrica, Sudán, Tanzania, Trinidad-Tobago, Uganda, Venezuela, Zambia, Zimbabwe.

Sobre hojas de algodón y de otras plantas causa manchas pardas pequeñas, circulares, con un borde estrecho púrpura. Estas manchas pueden expandirse y más tarde presentan centros agrietados, grises, secos. Sobre brotes, flores y cápsulas se forman lesiones redondas, pequeñas, sobre áreas glandulares, alternando con los puntos de inserción de las brácteas sobre el receptáculo (según ELLIS 1971).

Alternaria porri (Ellis)Cif. (Fig. 4/1)

J. Dep. Agric. P. Rico 14: 30. 1930.

Syn.: Macrosporium porri Ellis, Grevillea 8: 12. 1879.

Colonias dispersas. Micelio mayormente inmerso en el sustrato. Conidióforos solitarios o en grupos, rectos o flexuosos, a veces geniculados, pardo pálidos a pardos, hasta de 100 μm de largo por 4-8.5 μm de grueso, con una a varias cicatrices conidiales. Conidios usualmente solitarios, rectos o curvos, obclaviformes, o con el cuerpo del conidio elipsoidal, adelgazando hasta el cuello, pardo pálidos a pardo dorados, con 8-11 septos transversales y con uno a varios septos longitudinales u oblicuos, de 90-270 μm de largo por 12-18.5 μm de grueso en la parte más ancha; cuello flexuoso,

pálido, aproximadamente igual o más corto o más largo que la longitud del cuerpo, de 2-4 μm de grueso.

Hábitat: Sobre Allium cepa L. y Allium sativum L.

Material colectado en Cuba: Prov. Granma, Media Luna (IMI-119394); Prov. de Guantánamo, Mata Abajo, col. R. URTIAGA 1960. Prov. Ciudad de la Habana, Estación Agronómica de Santiago de las Vegas, col. R. TEXIDOR, 13. I. 1961, Herbario CH. BARKER (SV) HAC. Prov. Habana, Catalina de Güines, col. H. KREISEL 1969. Ver también SEIDEL (1976) y ARNOLD (1986).

Distribución: Alemania, Argentina, Australia, Brasil, Bulgaria, Canada, Colombia, Cuba, Dinamarca, Egipto, EE.UU., Etiopia, Filipinas, Ghana, Holanda, Honduras, Hong Kong, India, Irak, Israel, Jamaica, Japón, Kenya, Malasia, Malawi, Mauritania, Nicaragua, Nigeria, Nueva Zelanda, Paquistán, Polonia, Portugal, Puerto Rico, Rumania, Sabah, Salvador, Tailandia, Taiwan, Tanzania, Uganda, URSS, Venezuela, Vietnam, Yugoslavia, Zambia, Zimbabwe.

Sobre varias especies de Allium causa "mancha o pústula púrpura". Las manchas de las hojas son a menudo elípticas, grandes y coloreadas con algún tono de púrpura, a veces con un borde ancho, pardo pálido o amarillo.

Alternaria raphani Groves et Skolko (Fig. 3/2)

Can. J. Res., Sect. C. 22: 227. 1944.

Syn.: Alternaria mattiolarum Neergaard? Danish species of Alternaria and Stemphylium p. 177, 1945.

Colonias dispersas. Micelio mayormente inmerso en el sustrato. Conidióforos sin ramificar u ocasionalmente ramificados, pardo oliváceos, septados, lisos, a veces abultándose ligeramente en el ápice y usualmente con una cicatriz conidial; hasta de 150 μm de largo por 3-7 μm de grueso. Conidios comúnmente en cadenas de 2-3, rectos o ligeramente curvos, obclaviformes o elipsoidales, generalmente con un cuello corto, pardo dorado oscuros a pardo oliváceos, con 3-7 septos transversales y varios septos longitudinales u oblicuos, constreñidos en los septos, lisos o a veces finamente verrugosos; de 50-130 μm de largo por 14-30 μm de grueso en la parte más ancha. Clamidosporas abundantes producidas en cultivos, al principio unicelulares, redondas y ya maduras irregulares y con muchas células, pardas.

Hábitat: Sobre Raphanus sativus L.

Material colectado en Cuba: Ver FERNÁNDEZ ROSEÑADA (1973), SEIDEL (1976) y ARNOLD (1986).

Distribución: Canada, Cuba, Dinamarca, Egipto, EE.UU., Grecia, Holanda, India, Japón.

Produce manchas negras, hasta de 4 mm de diámetro sobre cápsulas con semillas de rábano. También se ha reportado sobre Matthiola incana y otras crucíferas (según ELLIS 1971).

Alternaria ricini (Yoshii) Hansford (Fig. 10/1)

Proc. Linn. Soc. Lond. 1942--1943: 52, 1943.

Syn.: Macrosporium ricini Yoshii, Bull. Sci. Fak. terk. Kjusu Univ. 3: 327. 1929.

Colonias anfígenas. Micelio mayormente inmerso en el sustrato. Conidióforos solitarios o en grupos, erectos, sin ramificar, rectos o flexuosos, casi cilíndricos o bastante más gruesos hacia la base, pardo pálidos, septados, lisos, con una o varias cicatrices conidiales, hasta de 80 μ m de largo por 5-9 μ m de grueso. Conidios solitarios u ocasionalmente en cadenas de dos, rectos o curvos, obclaviformes, o con el cuerpo del conidio elipsoidal, adelgazando casi abruptamente hasta un cuello muy estrecho, pardo pálidos, pardo rojizos o pardo dorados, con 5-10 septos transversales y varios septos longitudinales y oblicuos, a veces constreñidos en los septos, lisos de 70-170 μ m de largo por 13-27 μ m de grueso en la parte más ancha; cuello sin ramificar, pálido, de longitud igual o dos veces más largos que el cuerpo, de 1-1.5 μ m de grueso.

Hábitat: Sobre hojas y cápsulas de Ricinus communis L.

Material colectado en Cuba: Ver FERNÁNDEZ ROSEÑADA (1973) y ARNOLD (1986).

Distribución: Bulgaria, Costa de Marfil, Cuba, EE.UU., Etiopía, India, Japón, Kenya, Malawi, Nueva Guinea, Paquistán, Rumania, Senegal, Sudán, Tailandia, Tanzania, Uganda, Vietnam, Zambia, Zimbabwe.

Sobre ricino a veces causa severos daños a los semilleros, hojas e inflorescencias. Las manchas de las hojas son irregulares, variables en tamaño, pero a menudo muy grandes, pardas, zonadas, rodeadas por un halo amarillo. Hay dos tipos de síntomas sobre cápsulas: uno que produce repentina marchitez, decoloración púrpura o pardo oscura, colapso del pedicelo, pocas semillas, fracaso de una dehiscencia normal, y otro caracterizado por un hundimiento unilateral de un área que gradualmente se agranda hasta cubrir la cápsula completa. Todos los racimos y primordios florales pueden morir. Es común la defoliación prematura (según ELLIS 1971).

Alternaria solani Sorauer (Fig. 11/1)

Z. Pflkrankh. 6: 6. 1896.

Syn.: Macrosporium solani Ellis et Martin, Am. Nat. 16: 1003. 1882. Colonias dispersas. Micelio mayormente inmerso en el sustrato. Conidióforos solitarios o en pequeños grupos, rectos o flexuosos, pardo pálidos o pardo oliváceos, septados, lisos, hasta de 100 μm de largo por 4.5-8.5 μm de grueso. Conidios usualmente solitarios, rectos o ligeramente flexuosos, obclaviformes o elipsoidales, con cuello muy largo, pardo pálidos, pardo dorado pálidos o pardo oliváceos, con 10-30 septos transversales y pocos o sin septos longitudinales u oblicuos, lisos, de 90-260 μm de largo por 8-16 μm de grueso en la parte más ancha; cuello de longitud igual o algo más que el cuerpo, flexuoso, pálido, a veces ramificado, de 2-4.5 μm de grueso.

Hábitat: Sobre hojas y frutos de Lycopersicum esculentum Mill., sobre Solanum tuberosum L., Solanum melongena L., Capsicum annuum L. y Datura suaveolens H.B.K.

Material colectado en Cuba: Prov. Granma, Bayamo, 1966 (URTIAGA 1986). Prov. Ciudad de la Habana, Estación Experimental Agronomica de Santiago de las Vegas. col. R. GONZÁLEZ Herbario CH. BAKER (SV) HAC. Prov. Habana, Catalina de Güines, col. H. KREISEL 1969. Ver también SEIDEL (1976) y ARNOLD (1986).

Distribución: cosmopolita.

Se encuentra mayormente sobre papa, tomate, berenjena y otras solanáceas y también ha sido reportada sobre otros hospederos. Causa el tizón temprano de las papas, afectando todas las partes sobre el terreno. Sobre hojas causa manchas redondas, ovales o irregulares, pardas a pardo oscuras, a menudoconcentricamente arrugadas.

Alternaria sonchi J. J. Davis, apud. J. A. Elliot (Fig. 11/2)

Bot. Gaz. 62: 416. 1916.

Colonias anfigenas. Micelio mayormente inmerso en el sustrato. Conidióforos solitarios o en grupos, rectos o flexuosos, a veces geniculados, pardo pálidos, pardo oliváceo pálido o pardos, septados, lisos, hasta de 75 μm de largo por 4.5-9 μm de grueso, con una o varias cicatrices conidiales. Conidios solitarios o a veces en cadenas muy cortas, rectos o curvos, obclaviformes o cónicos, con un cuello corto y grueso, con la base ampliamente redondeada, pardo pálidos, pardo dorados o pardo oliváceos, con 4-8 septos transversales y varios o sin septos longitudinales u oblicuos, a menudo constreñidos en los septos, lisos o finamente verrugosos, de 55-125 μm de

largo por 14-24.5 μm de grueso en la parte más ancha; cuello de 4-9.5 μm de grueso.

Hábitat: Sobre Sonchus sp. y Lactuca sativa L.

Material colectado en Cuba: Prov. Ciudad de la Habana, Estación Experimental Agronómica Santiago de las Vegas, col. A. VÁZQUEZ DIAZ, Herbario CH. BAKER (SV) HAC (KREISEL 1971). Ver también SEIDEL (1976) y ARNOLD (1986).

Distribución: Cuba, Chipre, EE.UU., Etiopía, Kenya, Inglaterra, Libia, Mauritania, Sudán, Uganda, Zambia.

Se ha reportado sobre hojas de distintas especies de Sonchus que incluyen S. oleraceus y S. asper y también sobre especies de Lactuca. Causa manchas más o menos redondas, pardo grisosas arriba, con un margen estrecho púrpura. (Según ELLIS 1971.)

Alternaria tenuissima (Kunze ex Pers.) Wiltshire (Fig. 8/2)

Trans. Br. Mycol. Soc. 18: 157. 1933.

Syn.: Helminthosporium tenuissimum Kunze en C. G. et T.F.L. Nees, Nova Acta Acad. Caesar. Leop. Carol. 9: 242. 1818; Persoon, Mycol. Eur. 1: 18. 1822.

Macrosporium tenuissimum Fr., Syst. Mycol. 3: 374. 1832.

Colonias dispersas. Micelio mayormente inmerso en el sustrato. Conidióforos solitarios o en grupos, sin ramificar o ramificados, rectos o flexuosos, más o menos cilíndricos, pardo pálidos, con una o varias cicatrices conidiales, septados, lisos, hasta de 105 μm de largo por 3.5-6.2 μm de grueso. Conidios solitarios o en cortas cadenas, rectos o curvos, obclaviformes, con el cuerpo del conidio elipsoidal, adelgazando gradualmente hasta el cuello que alcanza una longitud igual a la mitad de la longitud del conidio, pero usualmente más corto, de 2-4 μm de grueso, frecuentemente abultado en el ápice, de 4-5 μm de grueso, donde puede haber varias cicatrices, pardo pálidos a pardo dorado claros, generalmente con 3-8 septos transversales y varios septos longitudinales u oblicuos, ligeramente constreñidos en los septos o no constreñidos, usualmente lisos, a veces finamente verrugosos, de 20-85 μm de largo por 7-18.5 μm de grueso en la parte más ancha.

Hábitat: Sobre hojas de Paspalum fimbriatum H.B.K., de Xanthosoma sagittifolium Schott, de Musa paradisiaca L., de Lactuca sativa L., de Ipomoea batatas L., de Polyscias guilfoylei Bailey, sobre flores de Althaea rosea Cav., sobre frutos de Capsicum annuum L., sobre peciolo de la hoja muerta de Roystonea regia (H.B.K.) O.F.Cook., y tallos herbáceos muertos no identificados.

Material colectado en Cuba: Prov. Granma, Media Luna, 1966 (IMI-126078); Bayamo, 1966; Las Ovejas, Bayamo, 1966; Jardín Botánico de Granma, Los Mameyes, cerca de Guisa, col. V. HOLUBOVÁ JECHOVÁ, 16. V. 1985 (PRM). Prov. Las Tunas, El Congo, Puerto Padre, 1966. Prov. de Santiago de Cuba, Daiquirí, Parque Baconao, col. V. HOLUBOVÁ-JECHOVÁ, 26. V. 1985 (PRM).

Distribución: cosmopolita.

Extremadamente común y reportada sobre muchas plantas, usualmente más como un invasor secundario que como un parásito primario.

REFERENCIAS

- Arnold, G. R. W. (1986): Lista de hongos fitopatógenos de Cuba. Editorial Científico-Técnica. Ciudad de la Habana, Cuba, 206 pp.
- Ellis, M. B. (1971): Dematiaceous Hyphomycetes. Commonwealth Mycol. Inst. Kew, 608 pp.
- Ellis, M. B. (1976): More Dematiaceous Hyphomycetes. Commonwealth Mycol. Inst. Kew, 507 pp.
- Fernandez Roseñada, M. (1973): Catálogo de enfermedades de plantas cubanas ACC. Inst. Inv. Tropicales Serie Agrícola 27: 1-78.
- Kreisel, H. (1971): Clave y catálogo de los hongos fitopatógenos de Cuba. Centr. Inf. Cient. Tec. Univ. La Habana ser. 4, Cienc. Biol. 20: 1-104.
- Seidel, D. (1976): Lista preliminar de hongos fitopatógenos de Cuba. Edit. Pueblo y Educación, La Habana, 186 pp.
- Urtiaga, R. (inédito): Notas micológicas 1965-1967, en el archivo de la correspondencia del Ing. Julian ACUÑA; Biblioteca del Inst. de Ecol. y Sist. de la Acad. de Cienc. de Cuba.

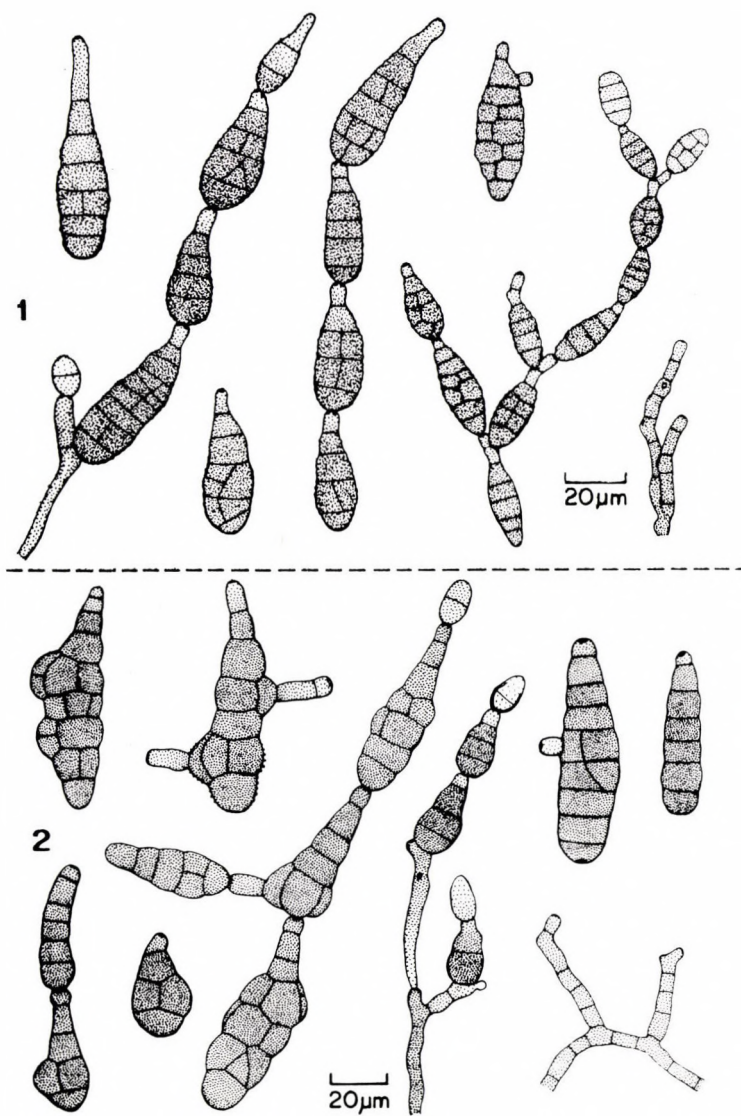


Fig. 1. 1) *Alternaria alternata* (Fr.) Keissler conidióforos y conidios;
 2) *Alternaria brassicicola* (Sch.) Willshire conidióforos y conidios

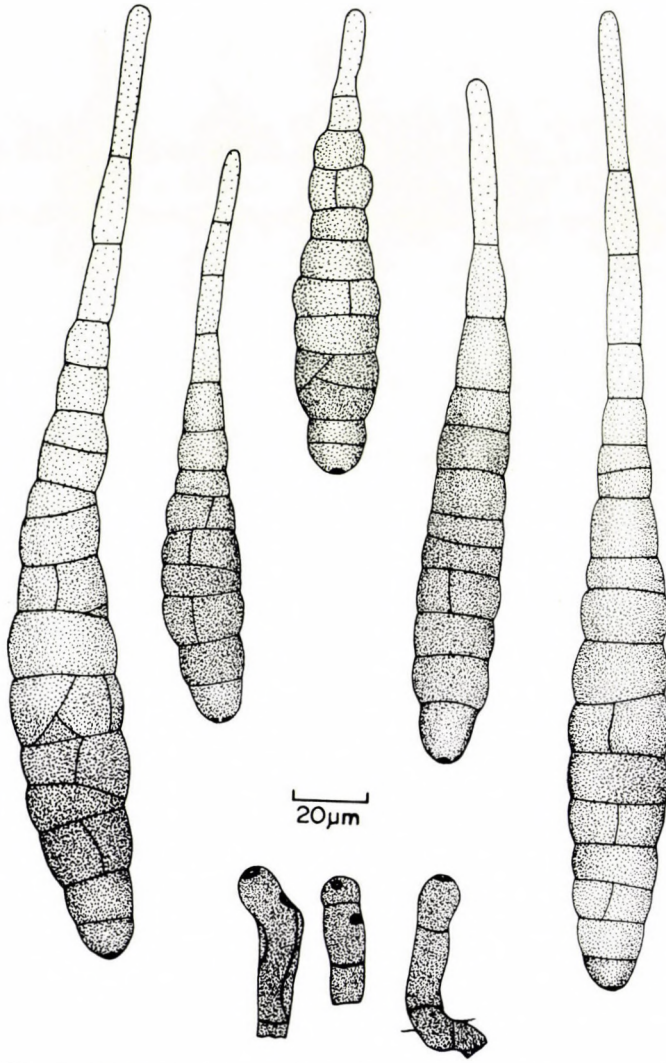


Fig. 2. *Alternaria brassicae* (Berk.) Sacc. conidióforos y conidios

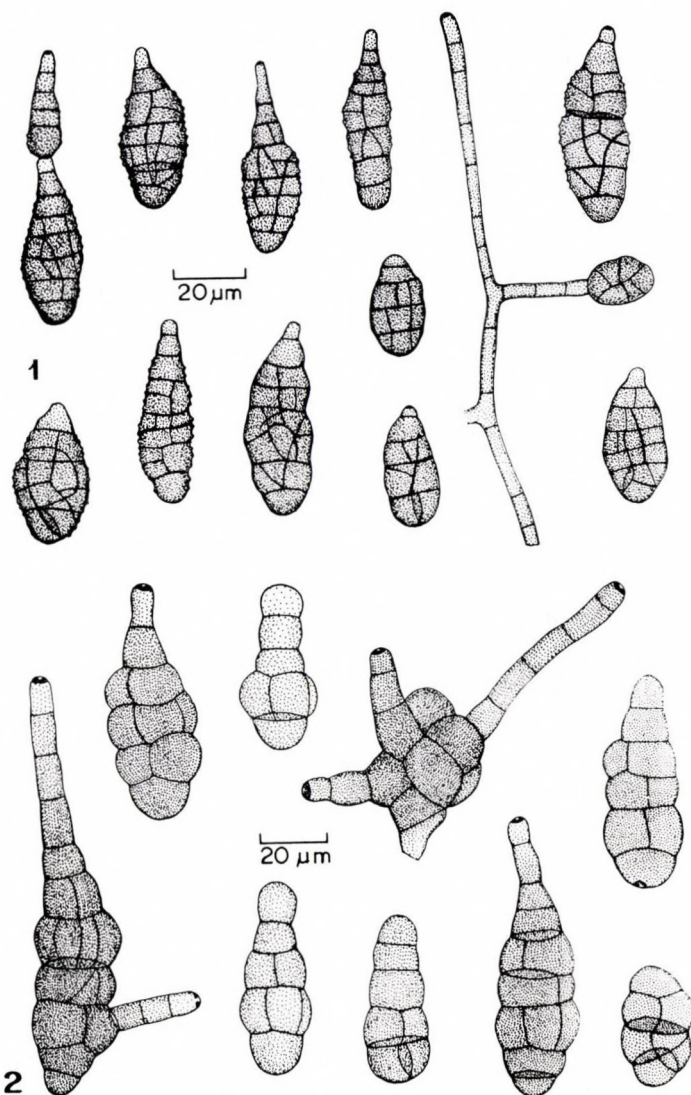


Fig. 3. 1) *Alternaria citri* Ellis et Pierce apud Pierce conidióforos y conidios;
 2) *Alternaria raphani* Groves et Skolko conidióforos y conidios

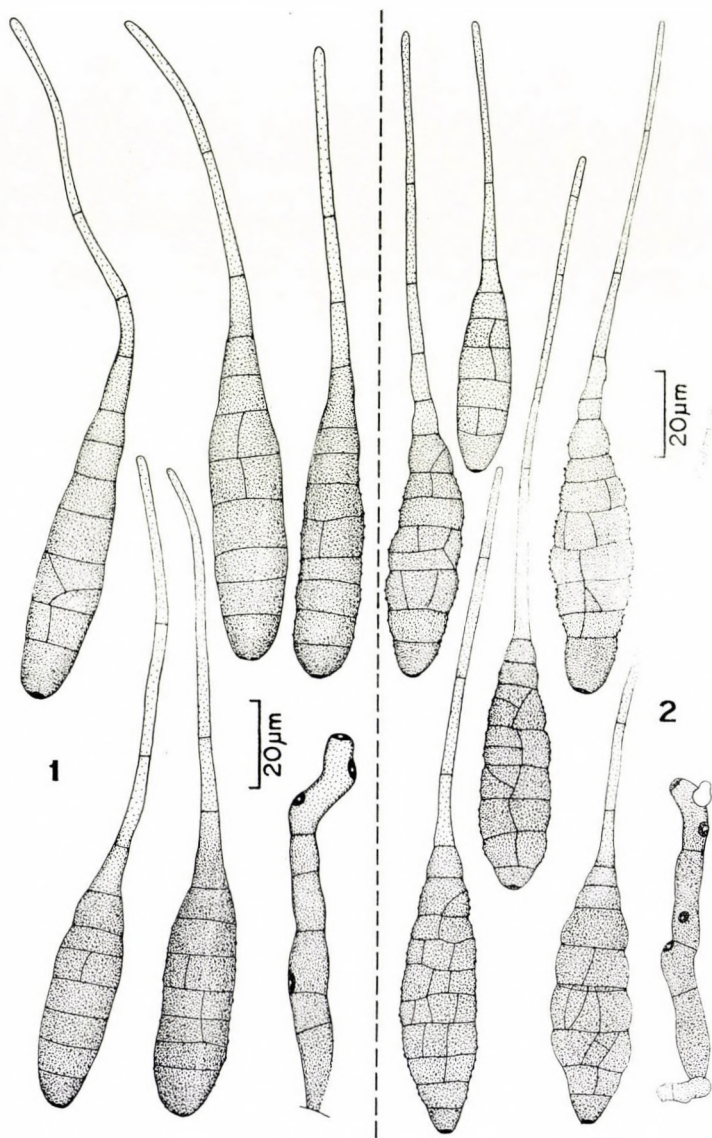


Fig. 4. 1) *Alternaria porri* (Ellis) Cif conidióforos y conidios;
 2) *Alternaria cucumerina* (Ellis et Everh.) Elliot conidióforos y conidios

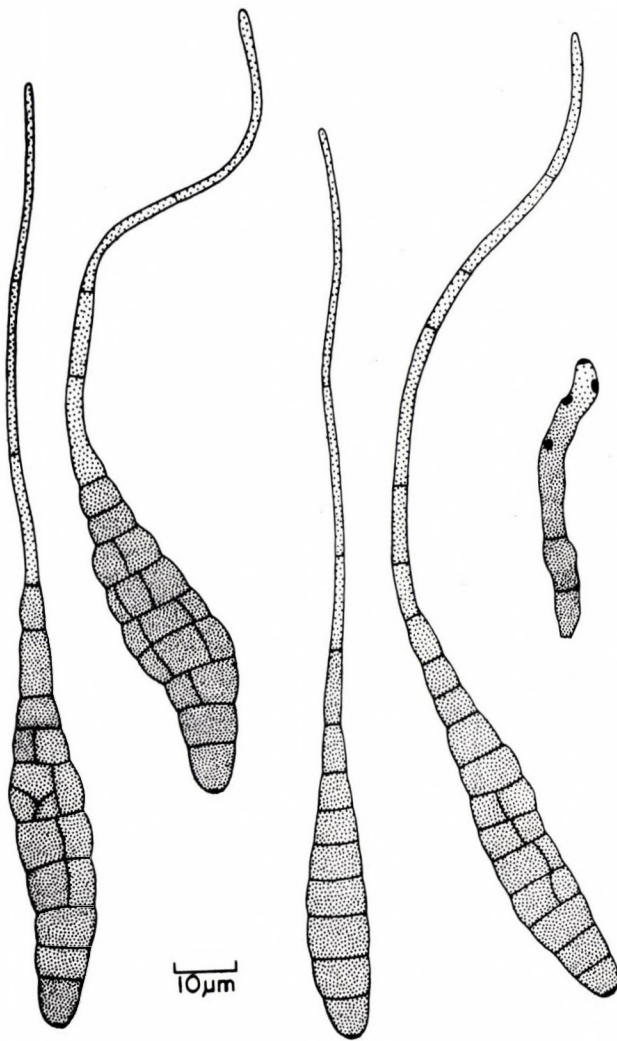


Fig. 5. *Alternaria crassa* (Sacc.) Rands conidióforos y conidios

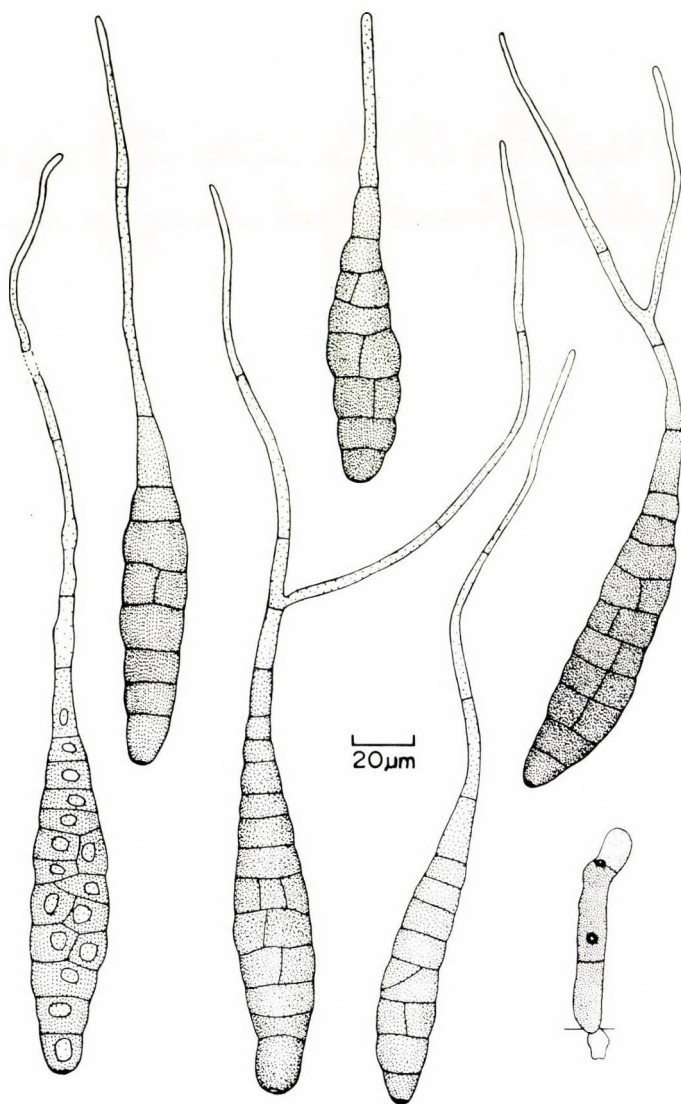


Fig. 6. Alternaria dauci (Kühn) Groves et Skolko conidióforos y conidios

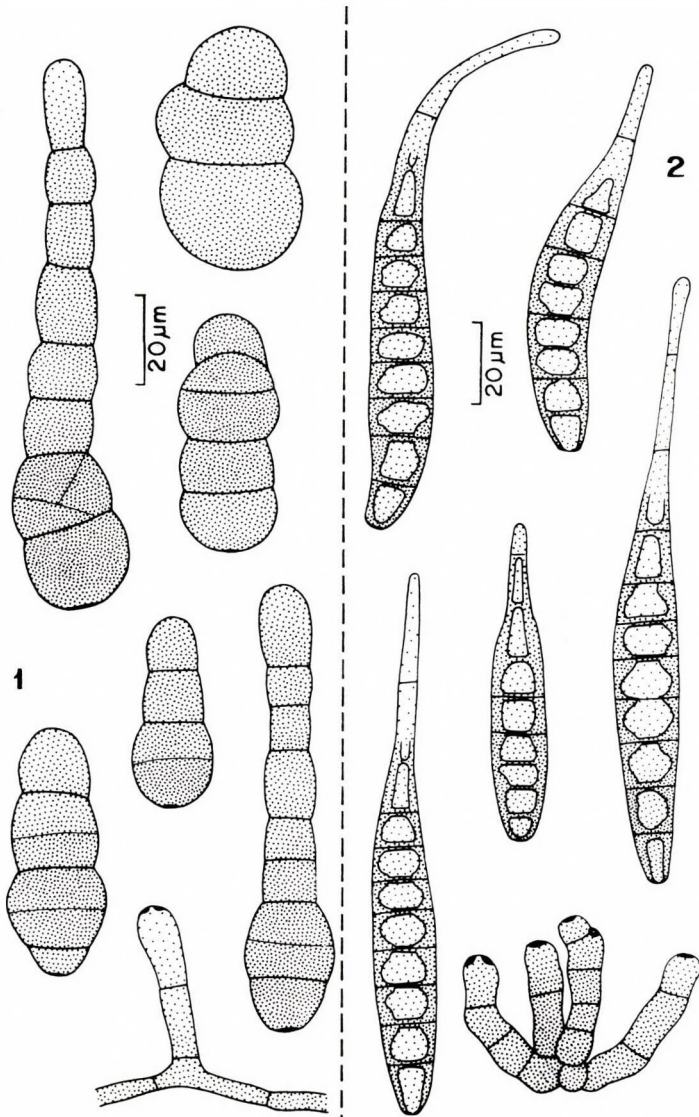


Fig. 7. 1) *Alternaria helianthi* (Hansf.) Tubaki et Nishihara conidióforos y conidios;
 2) *Alternaria gomphrenae* Togashi conidióforos y conidios

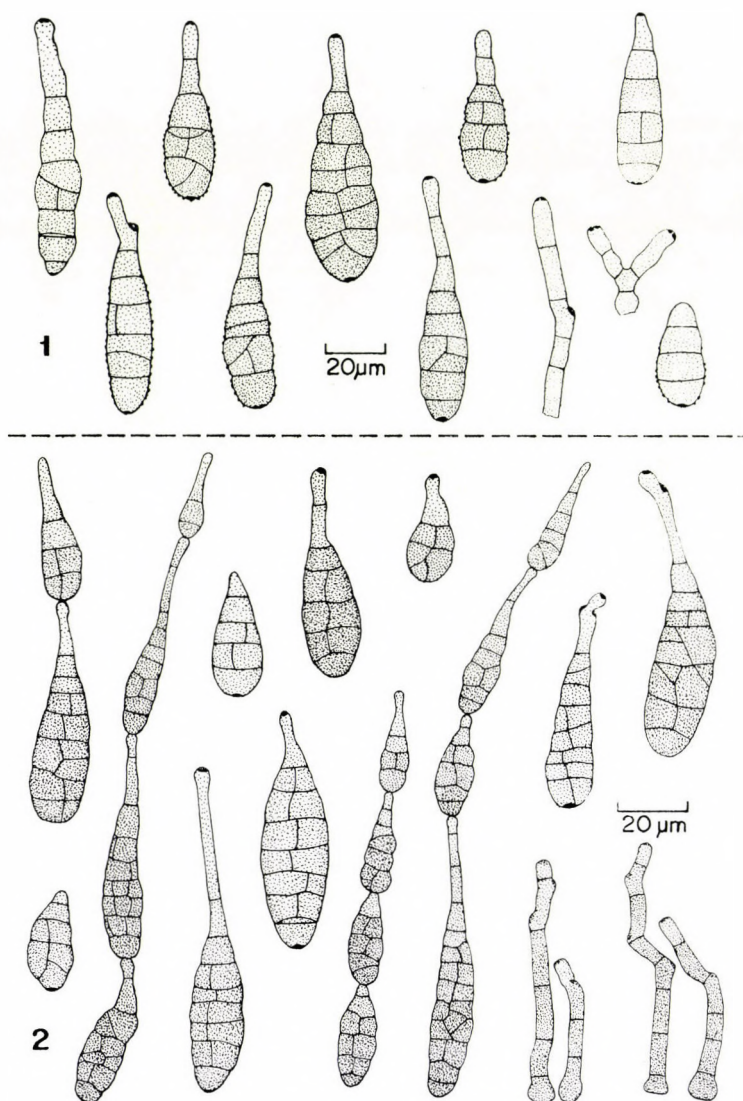


Fig. 8. 1) *Alternaria longipes* (Ellis et Ever.) Mason conidióforos y conidios;
 2) *Alternaria tenuissima* (Kunze et Pers.) Wiltshire conidióforos y conidios

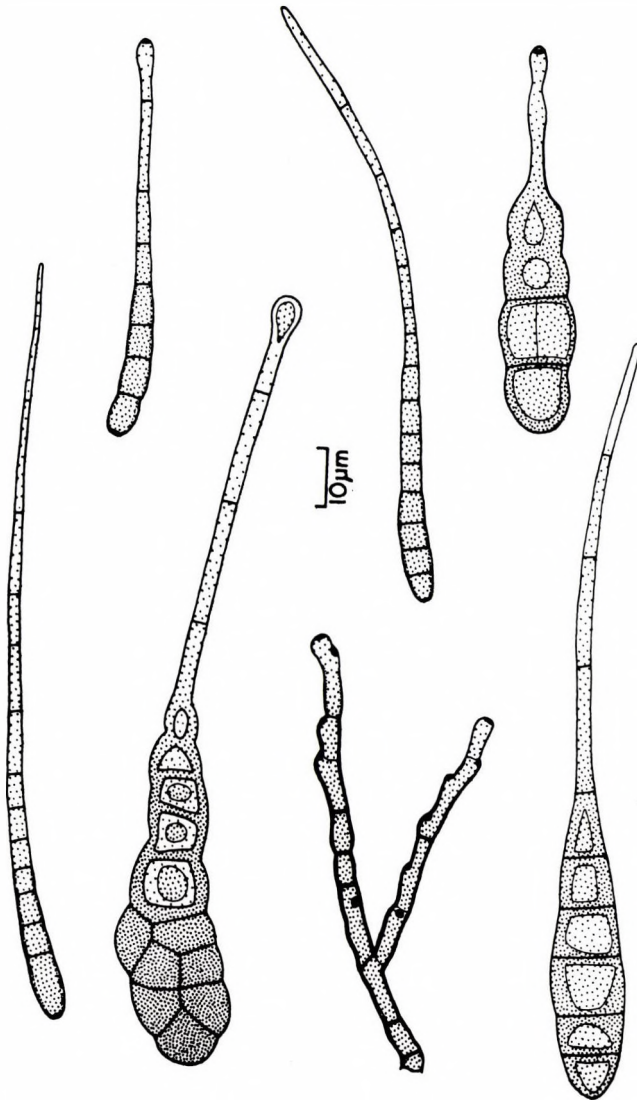


Fig. 9. Alternaria longissima Deighton et MacGarvie conidióforos y conidios

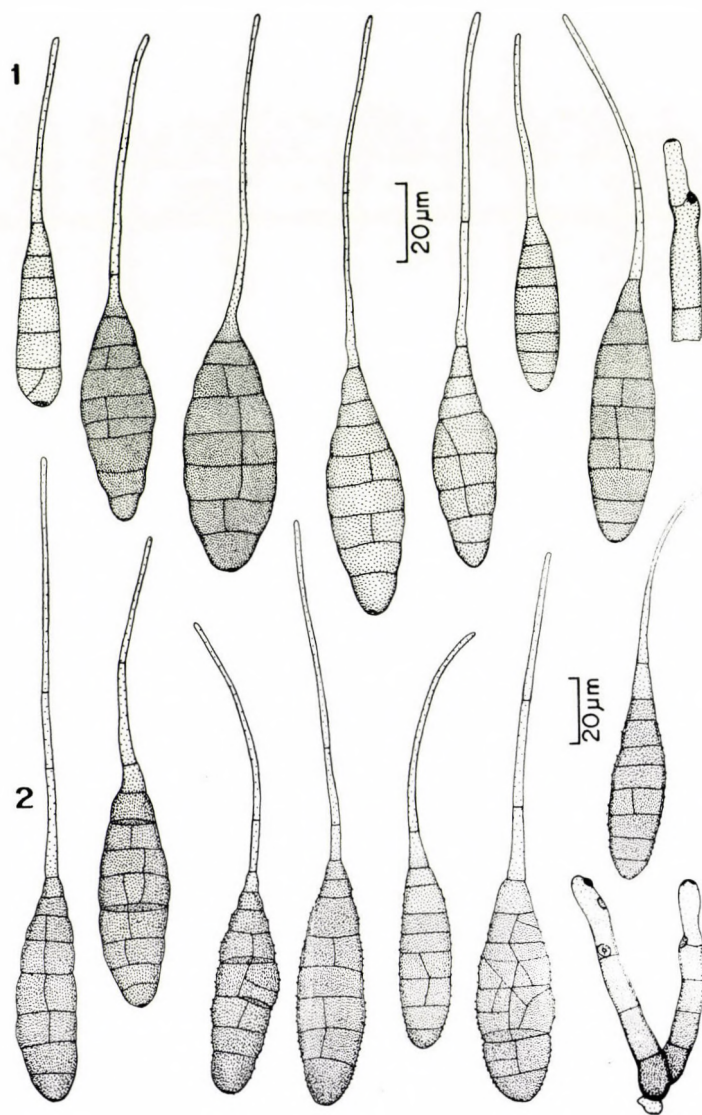


Fig. 10. 1) *Alternaria ricini* (Yoshii) Hansford conidióforos y conidios;
2) *Alternaria macrospora* Zimm. conidióforos y conidios

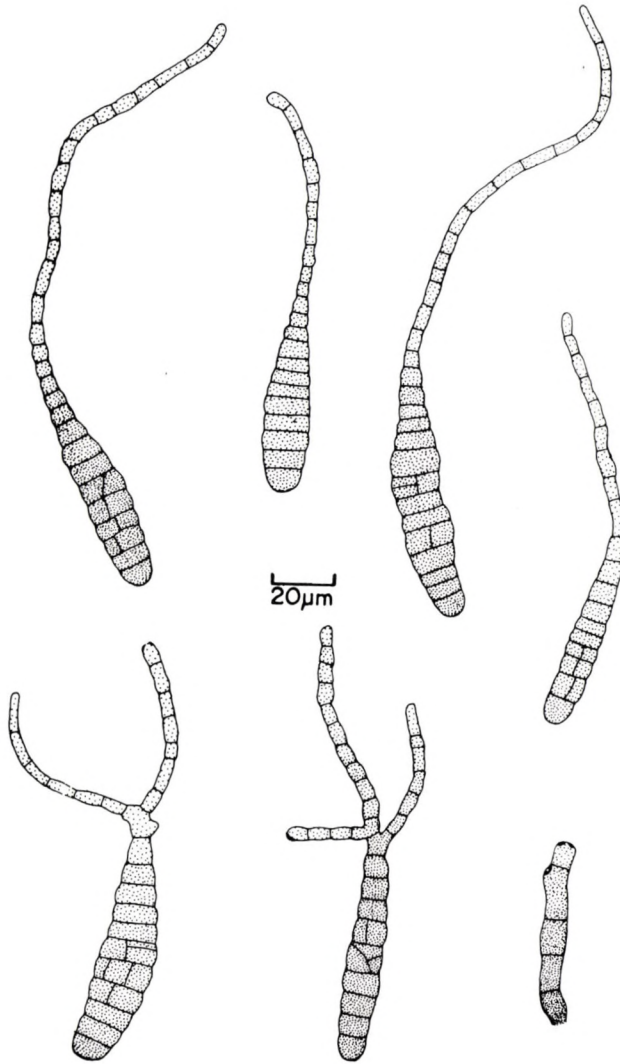


Fig. 11. *Alternaria solani* Sorauer conidióforos y conidios

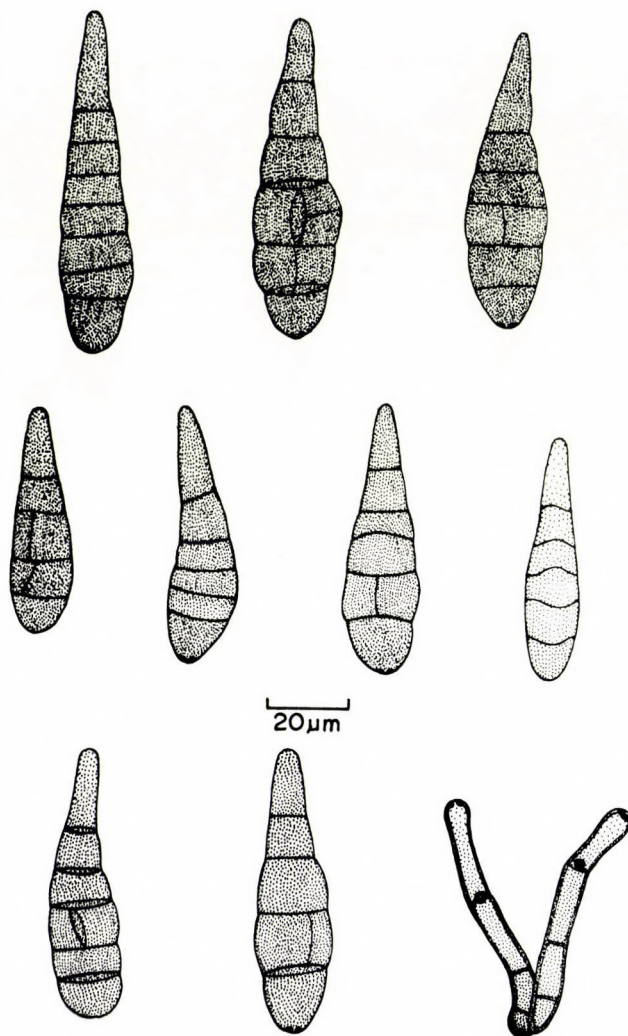


Fig. 12. Alternaria sonchi J.J. Davis apud J.A. Elliot conidióforos y conidios

NUEVOS O RAROS HIFOMICETES DE CUBA VII. ESPECIES ENTEROBLÁSTICAS

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(Recibido: 5 Noviembre 1991)

Nine species of hyphomycetes with enteroblastic conidiogenesis are described here. They were collected in several localities from Cuba. Three species are new for science and are conveniently illustrated. The remaining 6 taxa are new reports for our country and are scantily recorded in the world literature.

Introducción

Continuando el estudio taxonómico de los hifomicetes de Cuba y la serie de trabajos sobre táxones nuevos o raros encontrados recientemente en el país, presentamos en el presente artículo una relación de 9 especies con conidiogénesis enteroblástica, de las cuales tres constituyen nuevos táxones para la ciencia. Los ejemplares y tipos se encuentran depositados en el herbario de hongos del Instituto de Ecología y Sistemática de la Academia de Ciencias de Cuba (HACM).

Descripciones y Discusión

Corynespora gigaspora (Berk. et Broome) M.B. Ellis

Mycol. Pap. 65: 7. 1957.

Syn.: Helminthosporium gigasporum Berk. et Broome, J. Linn. Londres 14: 98. 1873.

Helminthosporium guaraniticum Speg., Annal. Soc. Cient. Arg. 22: 212. 1886.

Helminthosporium gigasporum Berk. et Broome subs. javanicum Penz. et Saccardo, Malpighia 15: 247. 1901.

Colonias dispersas, pardo oscuras a negras, pelosas. Micelio inmerso en el substrato, compuesto por hifas pardo pálidas a oscuras, de 2-6 µm de grueso. Estroma pardo oscuro, hemisférico o aplanado, parte superficial y

parte inmerso, pseudoparenquimático, de 20-60 μm de profundidad por 5-120 μm de ancho. Conidióforos solitarios o en pequeños fascículos, rectos o flexuosos, sin ramificar, pardo rojizo oscuros, septados, hasta con 4 proliferaciones cilíndricas sucesivas, 500-900 (-1230) μm de largo, por 11-23 μm de grueso, aguzándose hasta 10-13 μm en el ápice. Conidios solitarios, ocasionalmente en cortas cadenas, obclaviformes, rostrados, rectos o curvos, pardo dorado pálidos a oscuros, con 8-25 (-52) pseudoseptos (a veces con los septos muy ligeramente notables, lisos (82-) 100-150 (-270) \times 16-27 μm , truncados y 6-10 μm de ancho en la cicatriz basal oscura, 3.5-5 μm de ancho en el ápice.

Hábitat: Sobre ramas muertas no determinadas.

Material colectado en Cuba: Prov. de Sancti Spiritus, Sierra del Escambray, cerca de Jibacoa, col. V. HOLUBOVÁ-JECHOVÁ, 18. III. 1981 (PRM 825408); Prov. de Camaguey, Sierra de Cubitas, Hoyo de Bonet, col. J. MENA y V. MARTÍNEZ, 18. XII. 1985 (HACM 8035, 8039).

Distribución: Esta especie es conocida solo de Australia y Sri Lanka donde fue colectada sobre madera muerta (ELLIS 1957) y ahora de Cuba.

Corynespora kamatii (Vasant Rao) M.B. Ellis

More Dematiaceous Hyphomycetes, p. 376-377. 1976.

Syn.: Dendographium kamatii Vasant Rao, Curr. Sci. Bangalore 32: 473-474, 1963.

Colonias dispersas, pardo oscuras, pelosas. Micelio inmerso en el substrato, compuesto por hifas pardo pálidas a pardas, de 2.5-4.8 μm de grueso. Conidióforos en fascículos, estrechamente agrupados juntos para formar un sinema, hasta de 1400 μm de largo por 30-80 (-120) μm de ancho, alcanzando en la base un ancho de 180 μm ; los conidióforos individualmente son pardo pálidos con 1-2 proliferaciones percurrentes, 3-4 μm de grueso en el medio, ensanchándose hasta 5-6.5 μm en el ápice. Conidios obclaviformes, rectos o curvos, con 7-12 pseudoseptos, pajizos a pardo pálidos, lisis, (44-) 60-70 (-150) \times 10-13 μm con la cicatriz basal truncada, gruesa y oscura, de 4-5.5 μm de ancho.

Hábitat: Sobre ramas y tallos muertos de Cissus sp.

Material colectado en Cuba: Prov. de Santiago de Cuba, Sierra de la Gran Piedra, Reserva Natural Isabelica Norte, col. J. MENA, 22. V. 1985 (HACM 7639); y en el área del museo de la Isabelica, col. J. MENA, 23. V. 1985 (HACM 7695).

Distribución: La especie es conocida solo de la India donde fue colectada sobre tallos muertos de Vitis sp. (ELLIS 1976) y ahora de Cuba.

Craspedodidymum cubense Mena et Mercado sp. nov. (Fig. 1)

Coloniae effusae, brunneae. Mycelium in substrato immersum ex hyphis ramosis, septatis, brunneis, laevibus. Conidiophora magnifilamentosa, monofilamentosa, erecta, recta vel flexuosa, non ramosa, septata, plerunque percurrentes, brunnea vel atrobrunnea, apicem versus pallide brunnea, laevia; ad 460 μm longa 8-10 μm crassa ad basim, 5-7.5 μm ad apicem. Cellulae conidiogae phialidicae, percurrentes 18-26 μm longae, 8-12 (-14) μm crassae cum conspicuo collaretto 6-8 μm longo et 7.5-10.5 (-12.5) μm crasso. Conidia solitaria, subsphaerica, obovata vel pyriformia, brunnea vel atro-brunnea, aseptata, laevia, 11.5-15 x 10.5-13 μm .

Holotypus: HACM 7218. In rachide emortui Coccothrinaxis sp. Prov. Sancti Spiritus, Sierra del Escambray, Pico Potrerillo. col. J. MENA, 18. VII. 1984.

Colonias dispersas, pardas a pardo oliváceas. Micelio mayormente inmerso en el substrato, compuesto por hifas ramificadas pardas, septadas, lisas. Conidióforos sin ramificar, erectos, rectos o flexuosos, a veces percurrentes, pardo oscuros hacia la base y centro, tornándose pardo algo pálidos hacia el ápice, septados, lisos, hasta de 460 μm de largo por 8-10 μm de grueso en la base y 5-7.5 μm debajo de la célula conidiógena; hasta con 4 proliferaciones percurrentes (mayormente 2). Células conidiógenas fialídicas, de 18-26 μm de largo por 8-12 (-14) μm de grosor. Collarín grande de 6-8 μm de alto por 7.5-10.5 (-12.5) μm de grosor en la boca. Conidios solitarios, subesféricos, obovoides o piriformes, pardo oscuros, sin septos, lisos de 11.5-15 x 10.5-13 μm .

Hábitat: Sobre raquis de la hoja muerta de Coccothrinax sp.

Material colectado en Cuba: Prov. de Sancti Spiritus, Sierra del Escambray, ladera norte del Pico Potrerillo, col. J. MENA, 18. VII. 1984 (HACM 7218-Holótipo).

Distribución: Cuba.

Esta especie está relacionada con C. pulneyensis (Subramanian et Bhat 1987), pero se diferencia de ésta fundamentalmente por la forma y dimensiones de los conidios. C. cubense también se asemeja a C. abigianense (Lunghini et Onofri 1980) pero esta otra especie presenta los conidios obovoides o esféricos papilados y las células conidiógenas ocasionalmente polifialídicas con collarines más pequeños y estrechos.

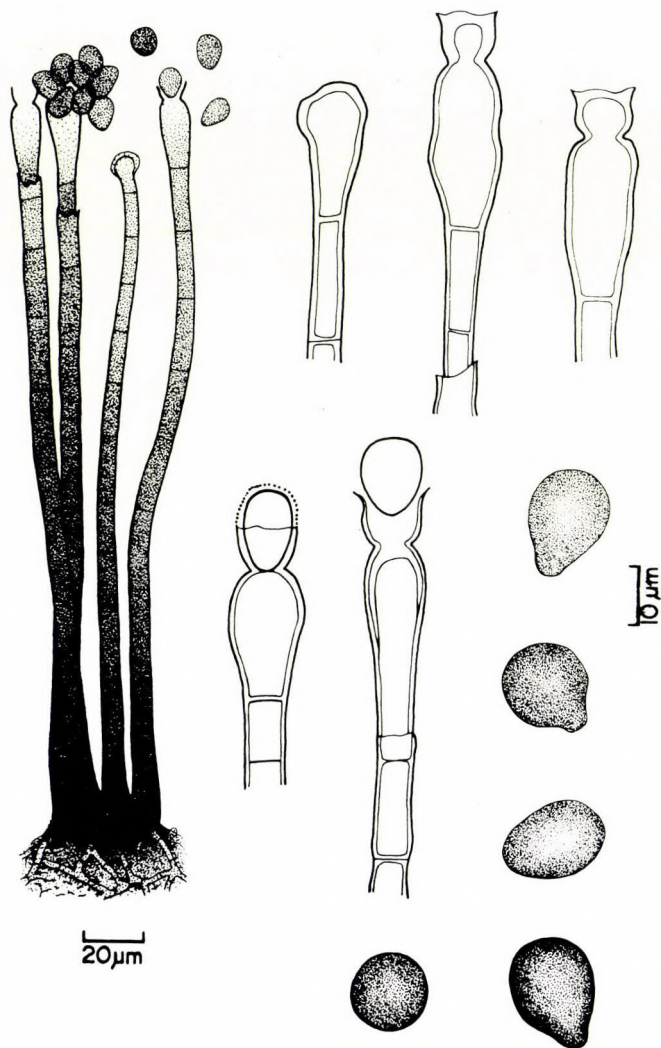


Fig. 1. Craspedodidymum cubense sp. nov. Conidióforos, fiálides con collarín y conidios

Exosporium pterocarpi M.B. Ellis

Mycol. Pap. 82: 36—37. 1961.

Syn.: Helminthosporium obovatum Masse, Kew Bull. p. 359. 1912.

Colonias dispersas, pardo pálidas a pardas, pelosas. Micelio inmerso en el sustrato, compuesto por hifas ramificadas, subhialinas a pardo oliváceo pálidas, septadas, lisas, de 2-6 µm de grosor. Estroma inmerso, pseudo-

parenquimático, pardo, hasta de 200 μm de ancho. Conidióforos cespitosos, no ramificados, rectos o flexuosos, en ocasiones con nudos o engrosamientos que se forman al proliferar los conidióforos, pardo rojizos, septados, lisos en casi toda su extensión, pero frecuentemente verrugosos hacia el ápice, hasta de 310 μm de longitud, pero generalmente más pequeños (100-160 μm), de 7-7.5 μm de grosor, de 9-14 μm en el ápice claviforme, percurrentes, con cicatrices poco notables. Conidios solitarios, rectos o curvos, obclaviformes a rostrados, subhialinos a pardo oliváceos, con una cicatriz oscura en la base, con 6-15 (-18) pseudoseptos, lisos, de 51-156 x 12-14 μm , de 3.5-7 μm de grosor cerca del ápice, de 4.5-5.5 μm en la base.

Hábitat: Sobre ramas muertas de Passiflora sp. y ramas y tallos herbáceos no identificados.

Material colectado en Cuba: Prov. de Sancti Spiritus, sur de Cayo Caguanes, col. J. MENA, 19. XII. 1984 (HACM 7288); Prov. de Camagüey, Sierra de Cubitas, Hoyo de Bonet, col. J. MENA y V. MARTÍNEZ, 18. XII. 1985 (HACM 8052); camino de Vilato a Lesca, col. J. MENA y V. MARTÍNEZ, 12. I. 1986 (HACM 8097); Prov. Granma, alrededores de Guisa, col. J. MENA, 15. V. 1985 (HACM 7469, 7470, 7472); col. V. HOLUBOVÁ-JECHOVÁ, 15. V. 1985 (PRM); Sierra Maestra, cerca del Zapote, col. J. MENA, 19. V. 1985 (HACM 7568); Prov. de Guantánamo, Río Duaba, col. A. MERCADO 31. V. 1985 (HACM 7812).

Distribución: Malasia (ELLIS 1961) y ahora en Cuba.

El hallazgo de Exosporium pterocarpi en Cuba constituye el segundo reporte a nivel mundial de esta especie, ya que con anterioridad solo se había encontrado sobre hojas de Pterocarpus en Malasia.

Los conidios de los ejemplares cubanos presentan mayor longitud y número de pseudoseptos que lo reportado por ELLIS (1961) para esta especie, de 51-156 μm de longitud y de 6-15 (-18) pseudoseptos por 60-115 (84) μm de longitud y de 3-12 pseudoseptos respectivamente.

Fuscophialis cubensis Mercado et Mena sp. nov. (Fig. 2)

Coloniae effusae, brunneae. Mycelium partim superficiale et partim in substrato immersum. Conidiophora solitaria, magnifilamentosa, monofilamentosa non ramosa, olivacea vel brunnea, septata, laevia, apicem versus pallide brunnea vel sub-hyalina 10-50 x 3.5-6 μm . Cellulae conidiogenae phialidicae, cylindricae vel conicae cum conspicuo collaretto ad 1.8 μm diametra. Conidia solitaria, obclaviformia, hyalina vel subhyalina, aseptata, laevia, 15-40 x 2-3 μm .

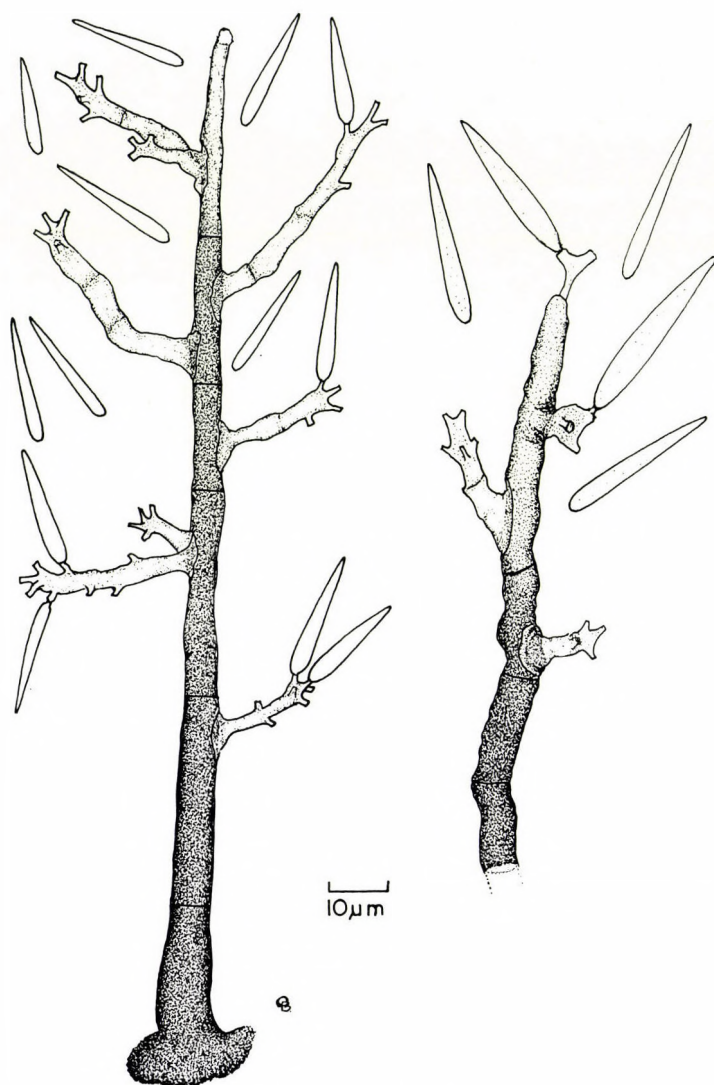


Fig. 2. *Fuscophialia cubensis* sp. nov.

Conidióforos sobre setas de *Beltrania rhombica* Piroz. Fiálides con collarin y fialoconidios aseptados

Holotypus: HACM 7666. Supra folia coacervata putrida et parasitica in setis *Beltraniae rhombicae* Piroz. in foliis *Calophylli antillani* Britton. Prov. Santiago de Cuba, Sierra de la Gran Piedra, Reserva Isabelica Norte, col. J. MENA, 23. V. 1985.

Colonias dispersas, pardas, poco notables. Micelio parcialmente superficial sobre el substrato. Conidióforos sin ramificar, solitarios, pardo oliváceos a pardo pálidos o pardos, septados, lisos, más pálidos hacia el ápice, de $10-50 \times 3.5-6 \mu\text{m}$. Fiálides cilíndricas o cónicas, con collarín definido hasta de $1.8 \mu\text{m}$ de diámetro. Conidios solitarios, obclaviformes, hialinos o subhialinos, sin septos, lisos, de $15-40 \times 2-3 \mu\text{m}$.

Hábitat: Sobre hojarasca y sobre seta de Beltrania rhombica Piroz. en hoja caída de Calophyllum antillanum Britton.

Material colectado en Cuba: Prov. de Santiago de Cuba, Reserva Isabelica Norte, Sierra de la Gran Piedra, col. J. MENA, 23. V. 1985 (HACM 7666-Holótipo); Isla de la Juventud, col. R. F. CASTAÑEDA, 25. X. 1984, C/84/99 INIFAT.

Distribución: Cuba.

Esta especie se diferencia de la especie tipo del género F. brasiliensis Sutton, por poseer conidios aseptados, obclaviformes y más largos. F. brasiliensis posee conidios con 1-3 septos, mayormente naviculares y que miden $18-28.5 \mu\text{m}$ de longitud (SUTTON 1977).

Helminthosporium novae-zelandiae Hughes

New Zealand J. of Botany. 18: 72. 1980.

Colonias dispersas, negras, pelosas. Micelio inmerso en el substrato, compuesto por hifas pardo pálidas, de $3.5-9 \mu\text{m}$ de grosor. Conidióforos solitarios o en ocasiones en pequeños grupos, no ramificados o muy raramente con una rama, rectos o flexuosos, pardo oscuros a negros en la base, pardos a pardo algo oscuros en el ápice, lisos, hasta de $510 \mu\text{m}$ de largo por $19-26 (-28) \mu\text{m}$ de grosor en la base, $9-11 \mu\text{m}$ justamente debajo del ápice, el cual a menudo está ligeramente abultado y alcanza hasta $12-13 \mu\text{m}$ de grueso. Células conidiógenas cilíndricas o claviformes. Conidios solitarios, obclaviformes a fusiformes, a veces algo rostrados, pardo dorados a pardo oscuros, pálidos hacia el ápice, 5-8 (6) septos transversales; de $52-94 \times 16-20 \mu\text{m}$, lisos.

Hábitat: Sobre ramas muertas no identificadas.

Material colectado en Cuba: Prov. de Santiago de Cuba, Sierra de la Gran Piedra, camino hacia el jardín de la Gran Piedra, col. J. MENA, 24. V. 1985 (HACM 7710).

Distribución: Nueva Zelandia (HUGHES 1980) y ahora en Cuba.

La revisión del material cubano de H. novae-zelandiae confirmó el criterio de HUGHES (1980) con relación a que esta especie se encuentra mejor

ubicada en el género Helminthosporium que en el género Dendryphiopsis, ya que la formación de ramas en los conidióforos es ocasional y se produce como resultado de la regeneración de éstos después de algún daño sufrido durante su desarrollo.

Polyschema cubensis Mena et Mercado sp. nov. (Fig. 3)

Coloniae effusae, brunneae vel atro-brunneae. Mycelium superficiale ex hyphis septatis, ramosis, subhyalinis vel brunneis, echinulatis vel laevis, 1-3 μm crassis. Conidiophora micronematica vel semimacronematica. Cellulae conidiogenae in mycelio vel conidiophoris incorporatae, terminales vel intercalares, subsphaericae, vel ampulliformes, brunneae, laeviae, 4-8.5 x 4.6 μm . Conidia cylindrica ellipsoidea vel claviformia, brunnea vel atro-brunnea, 1-3 (3) septata, laevia, 11-20 x 4.8-8 μm .

Holotypus: HACM 7550. In petiolo folii emortui Roystoneae regiae (H.B.K.) O.F. Cook. Prov. Granma, Sierra Maestra, Las Canarias prope Victorino, col. J. MENA, 18. V. 1985.

Colonias dispersas, pardo oscuras a negras, incospicuas. Micelio mayormente superficial, compuesto por hifas ramificadas, subhialinas, pardo pálidas, septadas, finamente equinuladas a lisas, de 1-3 μm de grosor, que dan lugar a conidióforos micronemáticos o semimacronemáticos. Células conidiógenas terminales e intercaladas, subesféricas o algo ampuliformes, pardas a pardo rojizas, lisas, con uno o dos poros notables, de 4-8.5 x 4-6 μm . Conidios cilíndricos, elipsoidales o claviformes, pardo rojizos a pardo rojizos algo oscuros, en ocasiones muy oscuros hacia el ápice, con 1-3 septos (mayormente 3) engrosados y oscuros, ligeramente constreñidos en los septos o hacia la parte central del conidio, lisos, de 11-20 x 4.8-8 μm .

Hábitat: Sobre el peciolo de la hoja muerta de Roystonea regia (H.B.K.) O.F. Cook.

Material colectado en Cuba: Prov. Granma, Sierra Maestra, Las Canarias, cerca de Victorino, col. J. MENA, 18. V. 1985 (HACM 7550-Holótipo).

Distribución: Cuba.

Este hongo presenta cierta relación con P. clavulata (Cke. et Harkn.) M.B. Ellis, la única especie de este género que tiene los conidios lisos, pero se pueden diferenciar porque los conidios de P. cubensis son más pequeños y tienen menor número de septos.

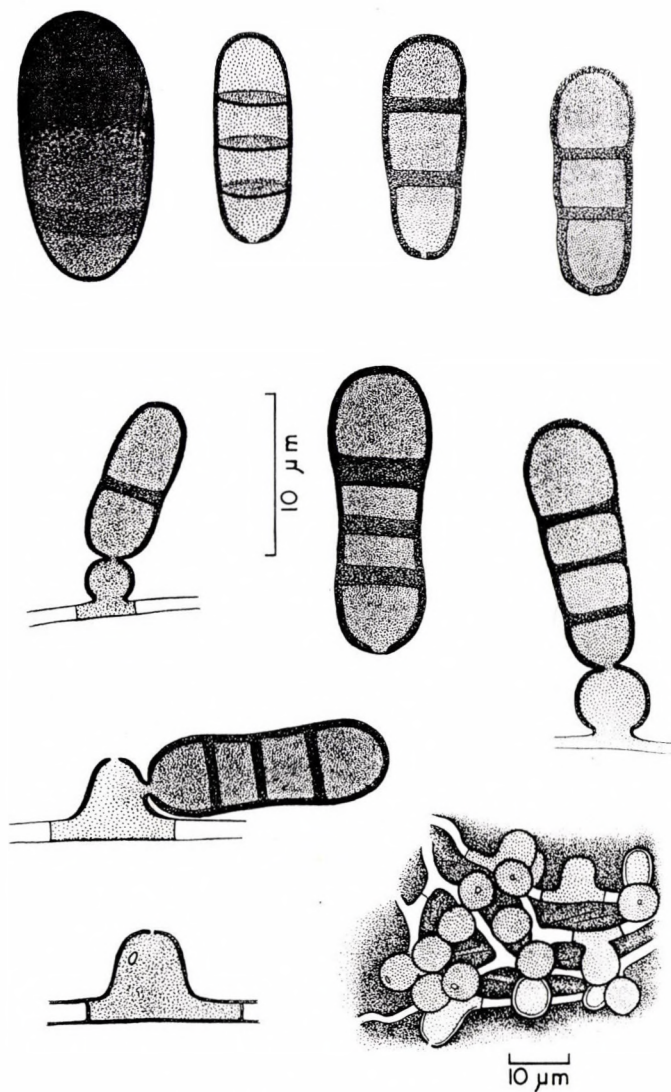


Fig. 3. *Polyschema cubensis* sp. nov.

Conidióforos micronemáticos, células conidiógenas discretas subesféricas y fragmoconidios con 1-3 septos transversales gruesos y oscuros

Virgatospora echinofibrosa Finley

Mycologia 59: 538--541. 1967.

Colonias dispersas, grises a pardo negruzcas. Micelio inmerso en el substrato. Conidióforos sinemáticos. Sinemas erectos, rectos o flexuosos, hasta de 1200 μm de alto por 40-75 μm de grueso, expandiéndose en el ápice y hacia la base hasta 140 μm de grueso, con una cabeza negra mucilaginosa. Filamentos del sinema subhialinos a pardos, ramificados hacia sus ápices, septados lisos o finamente equinulados, de 1.5-3.5 μm de grueso. Fiálides de 10-28 x 3-4.5 μm . Conidios rectos o curvos, elipsoidales, fusiformes o limoniformes, papilados en cada extremo, grises a pardo oscuros, con 3 septos transversales, a menudo con estriaciones longitudinales tenues de 35-48 x 10-16 μm , agrupados en cabezas mucilaginosas negras.

Hábitat: Sobre bejuco seco, ramas muertas y hojas muertas no identificadas.

Material colectado en Cuba: Prov. de Sancti Spiritus, Sierra del Escambray, ladera norte del Pico Potrerillo, col. J. MENA, 18. VII. 1984 (HACM 7206); Prov. de Camagüey, Sierra de Cubitas, Los Paredones, col. J. MENA, 17. XII. 1985 (HACM 8013, 8020); Prov. Granma, Los Mameyes, Guisa, Jardín Botánico de Granma, col. J. MENA, 16 y 17. V. 1985 (HACM 7493, 7498, 7499, 7543); Prov. de Guantánamo, márgenes del río Toa, cerca de Baracoa, col. A. MERCADO, 29. V. 1985 (HACM 7778).

Distribución: Cuba, Malaisia, Nueva Guinea, Panamá, Sierra Leona (ELLIS 1971).

La colección cubana difiere de la descripción de ELLIS (1971) porque los conidios presentan estriaciones longitudinales muy tenues, mientras que en la descripción e ilustración de Ellis son muy claras y notables.

Zanclospora brevispora Hughes et Kendrick

New Zealand J. Bot. 3: 156. 1965.

Colonias compactas cuando hay fuerte esporulación, grisáceas, aterciopeladas, o dispersas y algo pelosas cuando hay poca esporulación. Micelio parcialmente superficial y parcialmente inmerso en el substrato. Conidióforos sin ramificar, erectos, rectos o flexuosos, pardos, algo mas pálidos hacia el ápice, septados, lisos, de 150-250 μm de largo por 6-8.5 μm de grueso. Fiálides ordenadas en verticilos de 2-7 hacia la parte superior del conidióforo, justo debajo del ápice estéril, de 8-12 x 2-3.5 μm . Con un collarín en forma de embudo que mide 1.2-2 μm de ancho por 1-1.5 μm de profun-

dad. Conidios usualmente curvos, estrechamente obovoides o algo alantoides, hialinos, de $4.5-7 \times 1.5-2 \mu\text{m}$.

Hábitat: Sobre ramas muertas no identificadas.

Material colectado en Cuba: Prov. de Santiago de Cuba, Sierra de la Gran Piedra, Reserva Isabelica Sur, col. J. MENA, 24. V. 1985 (HACM 7778).

Distribución: Cuba, Nueva Zelandia.

Esta especie, descrita originalmente por HUGHES y KENDRICK (1965) de Nueva Zelandia, no ha sido reportada nuevamente, por lo que es ésta la segunda comunicación mundial de este hongo y la primera para el Hemisferio Occidental.

REFERENCIAS

- Ellis, M. B. (1957): Some species of Corynespora. Mycol. Pap. 65: 1-15.
- Ellis, M. B. (1961): Dematiaceous Hyphomycetes III. Mycol. Pap. 82: 1-55.
- Ellis, M. B. (1971): Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, 608 pp.
- Ellis, M. B. (1976): More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, 507 pp.
- Hughes, S. J. (1980): New Zealand Fungi 27: New species of Guedea, Hadrosporium and Helminthosporium. New Zealand J. Bot. 18: 65-72.
- Hughes, S. J., Kendrick, W. B. (1965): New Zealand Fungi 4. Zanclospora gen. nov. New Zealand J. Bot. 3: 151-158.
- Lunghini, D., Onofri, S. (1980): Craspedodidymum abigianense sp. nov., a new dematiaceous hyphomycete from Ivory Coast forest litter. Trans. Br. Mycol. Soc. 74: 208-211.
- Subramanian, C. V., Bhat, D. J. (1987): Hyphomycetes from South India I. Some New Taxa. Kavaka 15 (1, 2): 41-74.
- Sutton, B. C. (1977): Hyphomycetes from Eucalyptus Leaf Litter. Biol. Soc. Argent. Bot. 18: 158-160.

NEW NAMES AND NEW SPECIES IN THE FLORA OF
CUBA AND ANTILLES, IV

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Six new species of the flora of Cuba and Hispaniola are described and one sub-species. They are: Annonaceae: Xylopia acunae, Urticaceae: Pilea ophiticola, Rubiaceae: Chimarrhis ekmanii, Exostema pulverulentum, Rondeletia alaternoides ssp. brachyloba, Guettarda ekmanii, Asteraceae: Pentacalia acunae. Some new combinations are also published from the Chloranthaceae, Burseraceae, Myrtaceae, Lamiaceae, Rubiaceae and Asteraceae families.

Chloranthaceae

According to the monograph of A. C. TODZIA and E. C. WOOD jr. (The genus Hedyosmum (Chloranthaceae) in the West Indies. J. Arn. Arb. 69: 51--63. 1988) only 4 species exist in Cuba:

Hedyosmum nutans Sw.

H. grisebachii Solms-Laub. in DC.

H. domingense Urb.

H. subintegrum Urb.

H. leonis M.Vict. turned to be conspecific with H. grisebachii Solms-Laub. and H. crassifolium is considered as synonym of H. subintegrum Urb. The authors reduced the species H. cubense into a variety of the Hispaniolan H. domingense Urb. According to our concept about specific and infraspecific ranking H. cubense is really closely related to H. domingense and the morphological differences between them are more quantitative, than qualitative ones. Because of having a completely separated distribution area, we consider the two taxa as subspecies rather than varieties (See: BORHIDI: Acta Bot. Hung. 29: 182. 1983). Therefore:

Hedyosmum domingense Urb. ssp. cubense (Urb.) Borhidi stat. novus

Basionym: Hedyosmum cubense Urb. Repert. Spec. Nov. Reg. Veg. 24: 1. 1927. Type: EKMAN 5535 S!, Cuba: Sierra Maestra, Pico Turquino. Endemic to Cuba.

Annonaceae

Xylopia acunae Borhidi et Del-Risco sp. n.

Frutex vel arbor parva. Rami hornotini angulati, striati, adpresse albido- vel ferrugineo-setulosi, veteriores glabrescentes postremo glabri, teretes, brunnei, longitudinaliter et sparse transversaliter fissurati, lenticellis orbicularibus minutis satis dense obsiti. Stipulae e basi late triangulari abrupte acuminatae et subulatae, usque ad 1 mm longae. Folia 2-5 mm longe petiolata, petiolis minute puberulis, supra canaliculatis praedita, lamina elliptica vel oblongo-obovata, basi cuneata, apice rotundata vel obtusa, emarginata, 2-5 cm longa et 1-2.2 cm lata, nervo medio supra leviter impresso, apicem versus in sulco prominulo, subtus bene prominente, utrinque sparse puberulo vel glabrescenti, lateralibus utroque latere 4-6, supra tenuissime prominulis vel obsoletis, subtus manifestius prominulis, ante marginem arcuato-conjunctis et anastomosantibus, lamina utrinque opaca, glabra, utrinque prominenti-punctata, margine leviter crenulata, plana, chartacea. Flores in axillis foliorum 1-2 fasciculati. Bractee triangulari-ovatae, cca 1 mm longae, acutae, dorso carinatae et puberulae. Pedicelli 1-2 mm longi, puberuli. Alabastra tantum visa, oblongo-ovata, basi leviter dilatata, 2-2.5 mm longa, extus minutissime puberula vel glabrescentes. Cetera ignota.

Holotypus: 27266 HAC; Cuba centralis, Prov. Las Villas. Sierra de Escambray, Pico Potrerillo. Leg.: E. DEL-RISCO, 21. Nov. 1972.

Obs.: Inter omnes alias species cubanas foliis utrinque prominulo-punctatis, margine crenulatis et floribus glabrescentibus insignis.

Urticaceae

Pilea ophiticola Borhidi sp. n.

Suffrutex erectus, valde ramificatus, caulis 4-angulus, glaber, sine cystolithis. Folia inaequimagina, inaequaliter petiolata, stipulae minutae. Lamina folii rhombea, rariter ovata, 3-7 mm longa et 2-3 mm lata, apice rotundata, basi cuneata, rariter obtusa, margine integra, e basi 3-nervia, nervis lateralibus obsoletis vel inconspicuis, supra cystolithis linearibus numerosis praedita, subtus cystolithis nullis, utrinque glabra pergamacea. Inflorescentia mascula sessilis, folio multo brevior, floribus fasciculatis, 0.5-1.5 mm longe pedicellatis, perianthio glabro cystolithis suffulto, 3-4-lobulato, stamina 3-4, longe exserta, filamenta geniculata. Inflorescentia feminea axillaris, sessilis, foliis multo brevior, floribus fasciculatis, subsessilis, perianthio glabro, 3-lobulato, lobis valde inaequalibus, 2 majoribus 0.4-0.6 mm longis, 1 minore 0.2 mm longo, cystolithis obsitis. Achaenium album, glabrum, foveolatum, 0.8-1 mm longum et 0.5 mm latum.

Holotypus: BORHIDI 27777 HAC! Cuba Orientalis, Prov. Oriente, Sierra del Cristal, Rio Lebisa, al pie sur del Pico Cristal in alt. cca 900 m.s.m. Leg.: A. BORHIDI et M. A. VALES 12. apr. 1976. Isotypus: BP.

Obs.: Forma foliorum Pileam heteroneuram Griseb. revocat, quae a specie nostra foliis membranaceis, cystolithis linearibus utrinque dispositis, nervis lateralibus supra basim abeuntibus differt. Altera species, P. mayarensis Morton habitu similis, sed foliis aequalibus, ovatis, basi rotundatis vel cordatis, staminibus vix exsertis clare distinguitur.

Burseraceae

According to the palynological and morphological studies made by M. MONCADA on the Cuban Bursera species, it turned out that some of the Hispaniolan species of Bursera really belong to the genus Commiphora, formerly known as a palaeotropical genus. The following new combinations are suggested:

Commiphora ovata (Urb. & Ekm.)Borhidi comb. nova

Basionym: Bursera ovata Urb. & Ekm. Ark. f. Bot. 22 (8): 57. 1928. Hispaniola: Rep. Dominicana, Barahona, type: EKMAN, H 7001 S!

Commiphora spinescens (Urb. & Ekm.)Borhidi comb. nova

Basionym: Bursera spinescens Urb. & Ekm. Ark. f. Bot. 22 (8): 57. 1928. Hispaniola, Rep. Dominicana: Mare á Chat, Cabo Rojo, Haiti: Anse á Pitre, type: EKMAN H 6977, S!

Myrtaceae

Hottea Urb.

As it was supposed tentatively by URBAN (1923, 1926) leaf epidermis, ovary and embryological studies revealed, that Calycorectes ekmanii Urb. and the later described C. moana Borhidi and Muñiz belong to the genus Hottea (Myrtaceae). This genus was described by URBAN from the island of Hispaniola, based on the species Hottea miragoanae including further 4 Hispaniolan species to the genus.

Hottea is new to Cuba. Its generic diagnosis includes the following characters:

Arbustos o arbolitos con ramitas brevemente puberulas o glabras, hojas opuestas o ternadas, raramente alternas, con nervios laterales poco conspicuos o nulos. Flores 1-2 axilares, pediceladas; tubo de cáliz obovado, no estrechado o contraído sobre el ovario. Botón cerrado abriendo en 2, 4 o 5 lóbulos subiguales. Pétalos 1-5, desiguales, estambres en 2-4 series. Ovario bilocular, ovulos 6-14 pro celdas, afijos al septo medio o una columela central. Placenta poco evoluta. Baya 1-2-sperma. Semilla con testa cartácea. Cotiledones erguidos. 5 especies de Española y 2 de Cuba:

— Hottea ekmanii (Urb.)Borhidi comb. nova

Basionym: Calycorectes ? ekmanii Urb. Symb. Ant. 9: 110. 1923.

-- Hottea moana (Borhidi et Muñiz) Borhidi comb. nova

Basionym.: Calycorectes moana Borhidi et Muñiz Acta Bot. Acad. Sci. Hung. 21: 225. 1975.

Mosiera Small

This genus was proposed by SMALL in order to separate the neotrophic species of the genus Myrtus from the European Myrtus communis. Generic concept is very much debated in the Myrtaceae family. Morphological features of the embryo seem to gain an increasing taxonomic importance encouraging splitters to reestablish old generic names. BISSE (1985) published some new combinations transferring Cuban Myrtus species into Mosiera. Some of these needs correction because of violating the priority rule of the Code. Some further new combinations are added for complementing the list of the Cuban and Hispaniolan species. These are the followings:

Mosiera cabanasensis (Britt. et Wils.) Borhidi comb. nova

-- Basion.: Eugenia cabanasensis Britt. et Wils. Mem. Torr. Bot. Club 16: 88. 1920.

-- Syn.: Myrtus cabansensis (Britt. et Wils.) Alain Brittonia 20: 159. 1968. -- Eugenia buxoides Urb. Symb. Ant. 9: 99. 1923. -- Myrtus buxoides (Urb.) Burret Notizbl. Bot. Gart. Berlin 15: 483. 1941. -- Mosiera buxoides (Urb.) Bisse Rev. Jard. Bot. Nac. Cuba 6/3: 4. 1985.

Mosiera cabanasensis (Britt. et Wils.) Borhidi ssp. flavicans (Urb. et Ekm.) Borhidi comb. nova

-- Basion.: Eugenia flavicans Urb. et Ekm. in Urban: Symb. Ant. 9: 488. 1928.

-- Syn.: Mosiera flavicans (Urb. et Ekm.) Bisse Rev. Jard. Bot. Nac. Cuba 6/3: 5. 1985.

Mosiera cabanasensis (Britt. et Wils.) Borhidi ssp. pastelillensis (Urb.) Borhidi comb. nova

-- Basion.: Eugenia pastelillensis Urb. Symb. Ant. 9: 510. 1928. -- Mosiera flavicans (Urb. et Ekm.) Bisse ssp. pastelillensis (Urb.) Bisse Rev. Jard. Bot. Nac. Cuba 6/3: 5. 1985.

Mosiera calycolpoides (Griseb.) Borhidi comb. nova

-- Basion.: Psidium calycolpoides Griseb. Pl. Wright. I. Anal. Amer. Acad. Sci. 1860: 183.

Mosiera crenulata (Urb. et Ekm.) Borhidi comb. nova

-- Basion.: Psidium crenulatum Urb. et Ekm. in Urban Symb. Ant. 9: 460. 1928.

Mosiera del-Riscoi (Borhidi et Muñiz) Borhidi comb. nova

- Basion.: Myrtus del-Riscoi Borhidi et Muñiz Bot. Közlem. 64/3: 219. 1977.

Mosiera x miraflorensis (Borhidi et Muñiz) Borhidi comb. nova

- Basion.: Myrtus x mirafloresis Borhidi et Muñiz Acta Bot. Acad. Sci. Hung. 21: 227. 1975.

Mosiera tiburona (Urb. et Ekm.) Borhidi comb. nova

- Basion.: Eugenia tiburona Urb. et Ekm. Ark. f. Bot. 24 A. 4: 26. 1932.
-- Syn.: Myrtus tiburona (Urb. et Ekm.) Borhidi Acta Bot. Hung. 29: 186. 1983.

Mosiera tussackii (Urb. et Ekm.) Borhidi comb. nova

- Basion.: Eugenia tussackii Urb. et Ekm. Ark. Bot. 21 A 5: 37. 1927.
-- Syn.: Myrtus tussackii (Urb. et Ekm.) Burret Notizbl. Bot. Gart. Berlin 15: 483. 1941. -- Myrtus barkeri Urb. et Ekm. in schaedis.

Mosiera urbaniana Borhidi nomen novum -- Santo Domingo

- Myrtus flavicans Urb. et Ekm. Ark. Bot. 24 A (4): 16. 1931, non Mosiera flavicans (Urb. et Ekm.) Bisse Rev. Jard. Bot. Nac. Cuba 6/3: 5. 1985., nec Eugenia flavicans Urb. et Ekm. in Urban Symb. Ant. 9: 488. 1928.

Mosiera wrightii (Krug et Urb.) Borhidi comb. nova

- Basion.: Psidium wrightii Krug et Urb. in Englers Bot. Jahrb. 15/4: 570. 1892.

Lamiaceae

Micromeria Ekmaniana (Epling et Alain) Borhidi comb. nova

- Basion.: Satureja ekmaniana Epling et Alain in Alain Brittonia 20: 156. 1968.

Rubiaceae

Exostema pulverulentum Borhidi sp. n.

Frutex; rami hornotini teretes, rubelli, pilis brevissimis suavibusque pulverulenti, lenticellis oblongo-ellipticis obsiti, ad nodos compressi, veteriores striati et lenticellis dense dispositis rugulosi, cortice suberosi squamuliter delapsi. Stipulae interpetiolares deltoideae vel oblongo-triangulares, apice longe attenuatae et subulatae, 2-3 mm longae, basi ramorum lateralium in vaginam 1-2 mm longam connatae, puberulae. Folia oblongo-elliptica vel lanceolata, sub medio latissima, 5-10 mm longe petiolata, lamina basi cuneata vel obtusa, rariter rotundata, apice leviter acuminata et obtusa, 2.5-5 cm longa et 1.5-2.5 cm lata, nervo medio supra leviter prominulo, subtus prominenti, lateralibus utroque latere 4-6 utrinque tenuiter prominulis et ante marginem arcuato-conjunctis, lamina supra viridis, raphidibus minute

punctulata et pilis brevissimis erectis pulverulenta, subtus opaca, reticulata et pulverulenta, in axillis nervorum lateralium velutine domatiata, margine plana, membranacea vel chartacea.

Inflorescentiae corymbosae, multiflorae, terminales sessiles vel in axillis superioribus pedunculatae, 3-4 cm longae et 4-6 cm latae, dense breviterque pilosulae. Bractee membranaceae fimbriatae in lobulos lineares pulverulentosque dissectae, Rami corymbi 2-3.5 cm longi, pedunculi 1-1.5 cm longi puberuli, pedicelli 1-2 mm longi. Calycis tubus ellipticus cca 1 mm longus, puberulus, lobi (4)-5 lineares, obtusi, 0.2-0.3 mm longi inaequales puberuli. Corollae tubus 5-6 mm longus, sparse breviterque pulverulentus, 5-6 mm longus, lobi (4)-5, 1-2 mm longi, extus pulverulenti. Filamenta glabra, stamina exserta, antherae lineares 1.5-2 mm longae. Cetera non visa.

Holotypus: L. B. SMITH et al. 3268 (NY). Cuba: Prov. Santa Clara, Trinidad Mountains, San José, on low cliffs. Leg.: L. B. SMITH, A. R. HOGDON & F. GONZALEZ, 29. July, 1936. Isotypi: GH, VBI.

Obs.: Exostemae velutino Standl. et E. pervestito Borhidi & Fernandez affinis, sed species prima a specie nostra foliis ovatis, supra glabris et floribus 4-meris atque minoribus, secunda forma et pube foliorum bractearumque differt.

Chimarrhis ekmanii Borhidi sp. n.

Frutex vel arbor parva; ramuli hornotini cylindracei, glabri. Stipulae triangulares, e basi amplo abrupte longeque acuminatae et subulatae, apice acutae, 0.5-1.5 cm longae, utrinque glabrae. Petiolum 1-1.5 cm longum, anguste alatum, margine ferrugineo-puberulum, ceterum glabrum. Folia elliptica, basi longe attenuata et in petiolum protracta, apice acuta vel obtusiuscula, 4-7 cm longa et 1.8-3 cm lata, utrinque glabra, ad angulos nervorum lateralium setis rigidis albis valde domatiata, nervo medio subtus crassiuscule prominente, subtus prominulo, lateralibus utroque latere 5-9 in angulo 70-80° abeuntibus, utrinque prominulis, marginem versus arcuatis et obsoletibus, secundariis atque tertiariis reticulo leviter impresso formati, margine integra, plana, pergamacea. Inflorescentia axillaris, cymoso-corymbosa, 5-9 cm longa et 4-7 cm lata, pedunculus 3-5 cm longus, glaber, bractee triangulari-subulatae, 0.4-1 cm longae, rami corymbi et pedicelli ferrugineo-puberuli, ramuli terminales cymosi, 3-flori, flos centralis plerumque sessilis. Calycis tubus cum hypantio 5-6 mm longus, pars libera 1-2 mm longa, lobi minuti, late triangulares vel truncati, 0.1-0.3 mm longi, margine membranacei, glabri. Corolla 5-lobata, alba, 6-9 mm longa, tubus brevis usque ad 2 mm longus, lobi oblongo-obovati, apice rotundati, 5-8 mm longi, tubo 3-4-plo longiores; faux corollae sericeo-pubescentis. Stamina supra basem tubi corollini adnata, filamenta 1 mm longa, superne attenuata, dorso glabra, ventraliter densissime lanato-villosa. Antherae oblongae, dorsifixae, 4-5 mm longae, exsertae. Stylus 2-3 mm longus, apice capitatus, levissime 4-angulatus et brevissime bilobatus. Ovarium obtriangulatum, basi acutum, 3-4 mm longum. Discus annularis tenuis, superne glaber, breviter 2-fidus, capsula septicidalis 2-ocularis, 2-valvis, semina minuta, numerosa, globosa.

Holotypus: H 10389 (S) EKMAN; Hispaniola, Haiti; Massif de la Hotte, western group, Jérémie, Morne Pain de Sucre, in forest, in alt. cca 1400 m. s. m. Leg.: E. L. EKMAN, 22. 08. 1928.

Obs.: Chimarrhi cubensi Steyererm. affinis, a qua species nostra stipulis bracteisque longe subulatis, foliis atque inflorescentiis multo minoribus, limbo folii subtus domatiato, floribus fructibusque majoribus conspicue differt.

This taxon is obviously a small leaved, big flowered montane species, which substitute the lower montane Chimarrhis cubensis in the montane rain forest belt of the Massif de la Hotte. The species is very distinctive by its small, strongly domatiate leaves.

Rondeletia alaternoides A. Rich. in Sagra: Hist. Fis. Pol. Nat. CUBA XI. 1850: 13.

Type: LINDEN 2082 P!, Santiago de Cuba; isotypes: BM, NY. ssp. alaternoides (Fig. 1).

Inflorescentiae pauciflorae (5-9-florae), ferrugineo- vel albo-pilosae, folia non vel paullo superantes, saepe foliis breviores; lobulis calycis 1-1.5 mm longis, lineal-ovati vel linear-spathulati apice dilatati. Pedicelli florum terminalium 1-2 mm longi, erecti, adpresse ferrugineo-pilosi. Corolla extus non vel levissime adpresse sericeo-pilosa.

Inflorescencias paucifloras (5-9-floras) ferrugineo — ad albo-pelosas, no o muy poco sobrepasando la longitud de las hojas. Pedicelos terminales de 1-2 mm de largos, erguidos, ferruginoso-pelosos. Lóbulos del cáliz de 1-1.5 mm de largo, linear-ovales o linear-espátulados, el ápice obviamente ensanchado o redondeado. Area: Sierra de Cobre, Sierra de Nipe.

ssp. brachyloba Fernandez et Borhidi ssp. nova (Fig. 2).

(Syn.: Rondeletia subglabra auct. cub. sensu Alain Fl. Cuba 5: 45—46. 1962 pro maj.p., non Krug et Urb.)

Inflorescentiae multiflorae, laxe paniculatae, longitudinem foliorum duplo-triplo superantes, glabrescentes vel in fructu glabrae; pedicelli florum terminalium 2-5 mm longi, flexuosi; lobi calycini lineares, breves, usque ad 1 mm longi, apice obtusi vel rotundati, apice ipso non vel paullo dilatati.

Inflorescencias multifloras, laxamente apanojadas, 2-3-veces mas largar que las hojas, glabrescentes con pelos esparcidos y adpresos, o glabras en la fructificación: pedicelos terminales de 2-5 mm de largo, flexibles; lóbulos del cáliz cortos de hasta 1 mm de largo, lineales con ápice obtuso o redondeado, no conspicuamente dilatado. Area Sierras de Moa, Cristal y Baracoa, pinares y matorrales serpentinosos.

Holotypus: LEÓN 23054 HAC! Cuba; Prov. Holguin (Oriente). Pinares cerca de Punta Gorda, Moa. Arbusto de unos 3 m de alto, terreno de laterita. Coll.: LEÓN et CLEMENTE, 14. 07. 1947. Isotypes: GH; NY.

Specimina examinata: Sierra del Cristal: Cayo Verde, UO 2115, LÓPEZ FIGUEIRAS; — Subida al Cristal, ALAIN 5795; — Sierra de Moa: Mina Delta, CLEMENTE 4531; — Rio Cayoguán, CLEMENTE 3996, 5464, 5473, 6754; 4531, 6756, 7396, ALAIN, 848, 855; LEÓN 23124, 23130, 23136; — Mina Franklyn, Moa, CLEMENTE 3902, 3929; Rio Yagrumaje, WEBSTER 3751; — Punta Gorda, CLEMENTE 5367, 5473; LEÓN 20833, 23011, 23054; — Baracoa: Cañada de Quibiján, UO 2339 LÓPEZ FIGUEIRAS, sobre caliza: Abra del Rio Yumuri, ALAIN 7667; — Valle de Yumuri, LEÓN 18369; — Maisi, Cuesta de Piedra, LEÓN et SEIFRIZ, 18384; Maisi, LEÓN s.n.

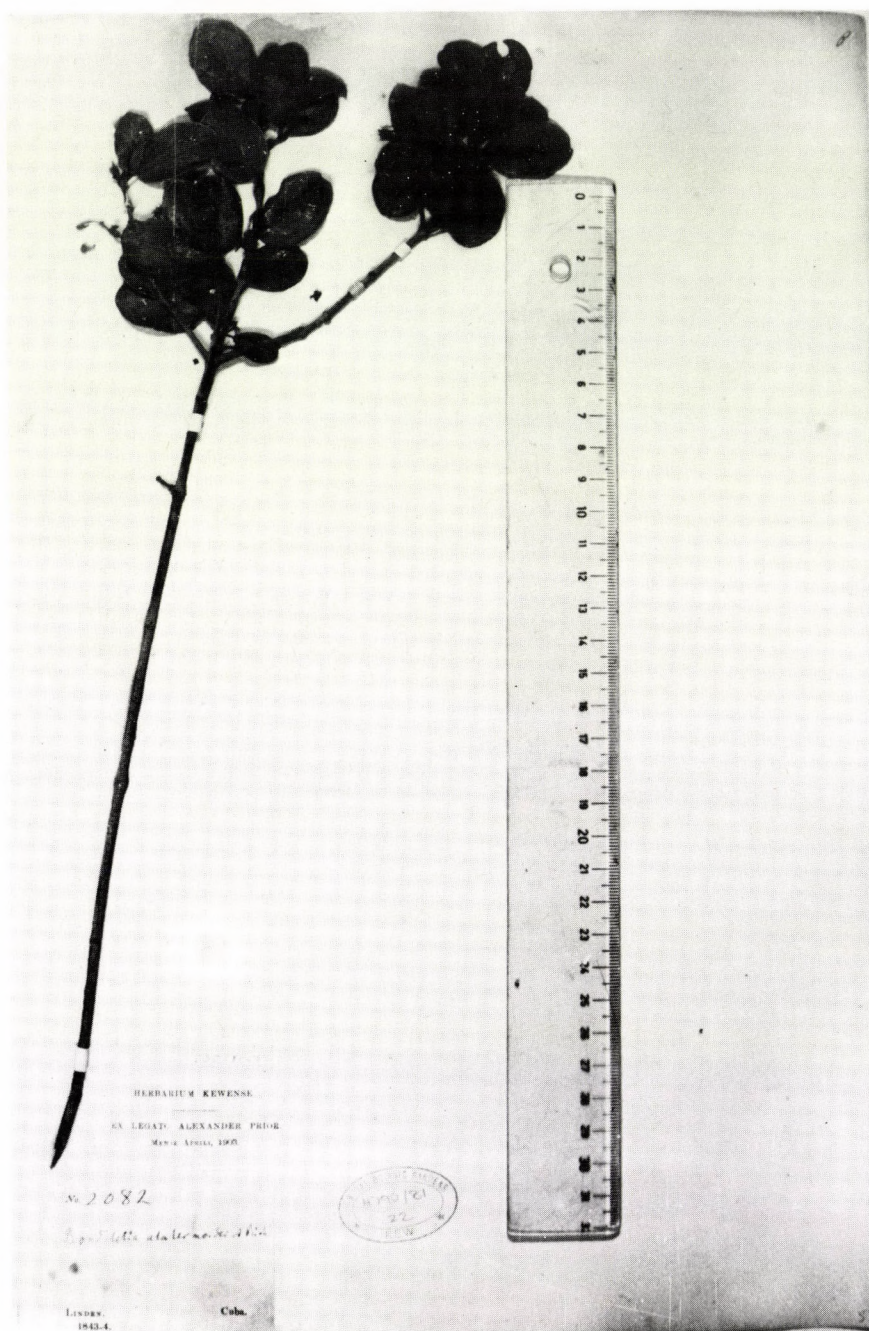


Fig. 1. Isotype specimen of Rondeletia alaternoides A. Rich. (LINDEN 2082, K)



Fig. 2. Holotype specimen of *Rondeletia alaternoides* A. Rich. ssp. *brachyloba* Fernandez et Borhidi (LEÓN & CLEMENTE 23054 HAC)



Fig. 3. Holotype specimen of *Rondeletia alaternoides* A.Rich. ssp. *myrtacea* (Standl. ex Britt.) Fernandez et Borhidi (LEÓN 11966, HAC)

ssp. myrtacea (Standl. in Britt.) Fernandez et Borhidi comb. nova (Fig. 3).

Basionym: Rondeletia myrtacea Standl. in Britt. in Bull. Torr. Bot. Cl. 53: 464. 1926.

Type: LEÓN 11966, Mesa de Prada, Jauco, NY; Isotypes: GH!; HAC!.

Inflorescencias cimoso-apanojadas, mayormente multifloras, 2-3 veces mas largas que las hojas, glabras, pedicelos de 3-10 mm de largo, lóbulos del cáliz oblongo espatulados de 1.5-2 mm de largo, contraídos en la base, mas largos del tubo. Area: Sierra de Imias, Sur de Baracoa.

Neolaugeria Nicolson

Because of the posteriority of the generic name Terebraria DC. ex Kuntze 1903 against Terebraria Greville 1864, and the generic name Laugeria Vahl ex Hook. 1873 against Laugieria Jacq. 1760 and Laugeria L. 1764 NICOLSON proposed Neolaugeria as a new generic name for the Antillean "Terebraria" species, including three species, L. apiculata, T. densiflora and T. resinosa. Two further species described by URBAN from Haiti were included into the synonyms of Neolaugeria apiculata (Britt. et Standl.)Nicolson. After having studied the EKMAN's types in Stockholm -- not seen by NICOLSON -- I am still considering that the leaf anatomical and epidermal characteristics of the Hispaniolan montane endemics do not permit to include them into the variability range of Neolaugeria apiculata. Therefore I suggest the re-validation of these species under the generic name of NICOLSON by creating the following new combinations:

Neolaugeria hotteana (Urb.)Borhidi comb. nova

Type: Haiti, Morne de la Hotte, Ekman H 601, S!

-- Basionym: Laugeria hotteana Urb. Ark. Bot. 20A (5): 58. 1926.

-- Syn.: Terebraria hotteana (Urb.)Alain Brittonia 20: 161. 1968. -- Neolaugeria apiculata (Britt. et Standl.)Nicolson. Brittonia 31: 120. 1979. pro min. p.

Neolaugeria lineolata (Urb.)Borhidi comb. nova

Type: Haiti, Massif de la Selle, Ekman H 1224, S!

-- Basionym: Laugeria lineolata Urb. Ark. Bot. 20A (5): 58. 1926.

-- Syn.: Terebraria lineolata (Urb.)Alain Brittonia 20: 161. 1968. -- Neolaugeria apiculata (Britt. et Standl.)Nicolson. Brittonia 31: 120. 1979. pro min. p.

Guettarda ekmanii Borhidi sp. n.

Arbor parva. Rami teretes, superne lateraliter compressi, apud cicatrices valde incrassati, hornotini dense adpresseque albo sericei, veteriores brunnei et glabrescentes, demum glabri, internodii 2-5 mm longi, apice dense foliosi. Stipulae interpetiolares triangulari-lanceolatae, 3-4 mm longae, apiculatae et ad 2 mm longe mucronato-subulatae, inter petiolos non conjunctae, dorso adpresse sericeae, valde diciduae. Folia 1-2 mm longe petiolata, late obovata, basi longe attenuata et acuta, in petiolum contracta; apice abrupte breviterque acuminata, mucronato-pungentia, 1-3 cm longa, 0.9-2 cm lata, nervo medio supra inferne impresso, superne prominulo, subtus valde prominenti, lateralibus utroque latere 6-9 utrinque prominulis et reticulato-anastomosantibus, ante marginem conjunctis, margine recurva, rigide coriacea, mucrone 1-3 mm longo. Lamina supra glabra, nitidula, subtus opaca, pilis sericeis adpressis sparse pilosula.

Flores solitariae ex axillis foliorum summorum prodeuntes, sessiles vel subsessiles, heterostyli, pedunculis usque ad 1.5 mm longis; prophylla 2 in vaginam 1-2 mm longam connata, superne libera, lineari-lanceolata, acuta, 1-1.5 mm longa, brunnea, extus pilosula, intus glabra, calycis tubus 2-3 mm longus, limbus cylindraceus, breviter adpresse sericeus, lobi 4, 0.2-0.3 mm longi, late rotundati. Corollae tubus cylindricus, 12-14 mm longus, medio 1.5-2.5 mm crassus, ad basim breviter attenuatus, basi extus breviter glaber, superne dense antrorso-pubescent, intus sparse pilosulus; lobi corollini 6, oblongo-obovati, 3.5-4 mm longi. Antherae fauci vel sub fauce insertae, lineares, sessiles, 2.5-3 mm longae. Discus cylindricus, adpresse pilosus, stylus brevior, 8-9 mm longus, pilosus, stigma crassus, breviter globosum, subintegrum; ovula in quoque loculo solitaria, ab apice pendula. Ovarium 5-loculare.

Holotypus: EKMAN H 15417 S!; Hispaniola, Santo Domingo; Cordillera Central; prov. de Samaná, Los Haitises, Boca del Infierno, limestone crags. 14. 06. 1930.

Obs.: Guettardae pungenti Urb. affinis, quae a specie nostra foliis ovatis, basi rotundatis vel obtusis, longius petiolatis, floribus tubo corollino retrorse piloso differt. Guettarda lamprophylla Urb. et Ekm. foliis supra lucidis, subtus nitidis, nervis lateralibus utroque latere 5-6, floribus usque ad 1 cm longe pedunculatis differt.

Psychotria L.

Psychotria patens Sw. is an endemic of Jamaica, does not exist in Cuba. It is to be deleted from the Flora of Cuba.

Psychotria cubensis (Steyerm.) Borhidi comb. nova

Basionym: P. deflexa DC. ssp. cubensis Steyerm. Mem. NY. Bot. Gard. 23: 505. 1972. -- Syn.: P. patens Alain Flora de Cuba 5: 105. 1962 non Sw.

Psychotria cuspidata Bredem. ex Roem. et Schult, is a plant of South America. It is to be deleted from the Flora of Cuba.

Psychotria didymocarpa (A. Rich.) Borhidi comb. nova

Basionym: Ronabaea didymocarpa A. Rich. Rubiac. 90. 1830; DC. Prodr. 5: 504. 1836; in Sagra: Hist. Fis. Pol. Nat. Cuba 11: 25. 1850.

Psychotria involucrata Sw. = P. officinalis Aubl. does not live in Cuba. The Cuban plant is: Psychotria hoffmannseggiana Muell.Arg. ssp. tribracteata (Wr. ex Griseb.) Borhidi comb. nova

Basionym: P. tribracteata Wr. ex Griseb. Cat. Pl. Cub. 1866: 137.

Psychotria nervosa Sw. ssp. rufescens (HBK.) Steyerl.

Area: Cuba, Bahamas, Puerto Rico, Central America, Colombia, Venezuela.

Psychotria sagraeana Urb. = P. carthaginensis Jacq.

Psychotria horizontalis Sw. Prodr. Veg. Ind. Occ. 1788: 44.

-- ssp. horizontalis: Hispaniola, Lesser Antilles

-- ssp. glaucescens (HBK.) Borhidi comb. nova -- Cuba

Basionym: P. glaucescens HBK. Nov. Gen. Spec. 3: 358. 1819.

Psychotria pendula (Jacq.) Urb. = P. guadalupensis (DC.) Howard

-- ssp. guadalupensis: Hispaniola, Lesser Antilles, Trinidad

-- ssp. tetrapyrena (Urb.) Steyerl. -- Cuba, endemic

Asteraceae

Pentacalia Cassini

The genus Pentacalia (Cassini Dict.Sc. Nat. 48: 461. 1827) has been resurrected by ROBINSON and CUATRECASAS (1978) mainly based on the section Streptothamni Greenm. of Senecio. The taxonomy of the tribe Senecioneae has been re-considered upon new generic alignments based on new microscopic histological and flower-morphological features (NORDENSTAM 1978). After having traced the generic limits of Pentacalia, a number of Central and South American species of Senecio were transferred to Pentacalia. Following ROBINSON's suggestion, three Jamaican taxa of the former Senecio were qualified as Pentacalia species and the closely related Cuban Senecio almironcillo was also transferred to Pentacalia by PROCTOR. According to ROBINSON and CUATRECASAS the most important generic features of Pentacalia are the 5-ribbed achene, enlarged cells of the anther collars and separate stigmatic lines, blunt tips of the style branches. Although many Cuban — and Jamaican — representatives of the genus Pentacalia differ from the typical species in having foveolate receptacles, white flowers, pilose or hirtulous achenes and corolla tube widened at the base, here the broader generic sense of Pentacalia (according to H. ROBINSON) is accepted. Further detailed studies of the Antillean species will declare, whether they have to be considered as separate section within the framework of Pentacalia or they would be more

properly classified into a separate Antillean genus, as it was the case of Odontocline and Jacmaia (NORDENSTAM 1978). At the first approach 13 Cuban and one Hispaniolan Senecio can be treated as taxa belonging to Pentacalia. One of them — Senecio almironcillo Gomez-Maza — was transferred to Pentacalia by PROCTOR (1982). The others are:

Pentacalia barahonensis (Urb.)Borhidi — Hispaniola

Basionym: Senecio barahonensis Urb. Ark. Bot. 23A (11): 91. 1931.

Pentacalia acunae Borhidi sp. n.

Frutex 1-2 m altus, rami angulati, longitrorse sulcati, dense lanato-tomentosi, postremo glabrescentes. Folia alterna, lanceolata, 3-6 cm longa et 0.7-1.2 cm lata, apice acuminata et plerumque acuta, basi longe cuneata et in petiolum 5-10 mm longum contracta, supra in statu juvenili arachnoideo-tomentosa, mox glabra, in statu matura opaca et papilloso-rugulosa, subtus persistenter albo-lanata, nervis utrinque inconspicuis, margine integra, revoluta. Inflorescentia terminalis, corymboso-cymosa, multicapitata. Capitula cylindracea, 6-8 mm longa, squamae involucri 5, lineari-lanceolatae, 5-6 mm longae, acutae, basi bullatae et brevissime connatae, apice obtusae vel apiculatae, basim versus arachnoideo-tomentosae, mox glabrescentes. Flores 5, corolla ochroleuca, 6-6.5 mm longa, tubus 4 mm longus, lobi corollae 2-2.5 mm longae, oblongo-ovatae. Antherae tubo corollae medio affixae, filamenta 1 mm longa, antherae 1.2-2 mm longae. Stylus 5 mm longus, glaber. Achaenia 4-angulata, ad angulos strigilloso-hirsuta, 3-3.5 mm longa. Pappi setis albis, 4-4.5 mm longis, corolla manifeste brevioribus.

Holotypus: ACUÑA 13412 SV in HAC. Cuba centralis, Prov. Las Villas, Sierra de Escambray, in saxosis calcareis montis Pico Potrerillo, in alt. 900-930 m.s.m. Leg.: J. ACUÑA, in Maio, 1939.

Specimina examinata: ALAIN 6386. Prov. Las Villas, Sierra de Escambray, cumbre del Pico Potrerillo. Leg. ALAIN 16. Julio, 1957.

Obs.: Pentacaliae almironcillo (Maza)Proctor affinis, quae a specie nostra foliis 4-10 cm longis, 1-2 cm latis, supra lucidis et laevissimis, capitulis 8-11 cm longis, corolla 7-8 mm longa, lobis corollae 3.5-4 mm longis, antheris tubo corollino sub medio affixis, filamentis 2-2.5 mm longis, achaenio 8-costato, setis pappi corolla aequilongibus differt.

Pentacalia carinata (Greenm.)Borhidi comb. nova — Cuba

Basionym: Senecio carinatus Greenm. Field Mus. Hist. Nat. 2 (8). 323. 1912. Type: SHAFER 4079. Sierra de Moa: Campo San Benito.

Pentacalia cubensis (Greenm.)Borhidi comb. nova — Cuba

Basionym: Senecio cubensis Greenm. Field Mus. Hist. Nat. 2 (8). 324. 1912. Type: SHAFER 4084. Sierra de Moa: Campo San Benito.

Pentacalia eriocarpha (Greenm.)Borhidi comb. nova — Cuba

Basionym: Senecio eriocarphus Greenm. Torreyia 13: 257. 1913.

Pentacalia leucolepis (Greenm.) Borhidi comb. nova -- Cuba

Basionym: Senecio leucolepis Greenm. Field. Mus. Hist. Nat. 2 (8). 324. 1912. Type: SHAFER 4146. Campo Toa.

Pentacalia moaensis (Alain) Borhidi comb. nova -- Cuba

Basionym: Senecio moaensis Alain Contrib. Ocas. Mus. Hist. Nat. La Salle, 18: 11. 1960. Type: LEÓN & CLEMENTE 23203, Sierra de Moa: Mina Johnston.

Pentacalia pachypoda (Greenm.) Borhidi comb. nova -- Cuba

Basionym: Senecio pachypodus Greenm. Field. Mus. Hist. Nat. 2 (8). 325. 1912. Type: SHAFER 8186, Sierra de Moa: Campo La Gloria.

Pentacalia polyphlebia (Griseb.) Borhidi comb. nova -- Cuba

Basionym: Senecio polyphlebius Griseb. Plant. Wright. Anal. Amer. Acad. 515. 1860. Type: CH. WRIGHT 329, Monteverde, Guantánamo.

Pentacalia saugetii (Alain) Borhidi comb. nova -- Cuba

Basionym: Senecio saugetii Alain Contrib. Ocas. Mus. Hist. Nat. La Salle, 18: 12. 1960. Type: LEÓN 12196. Sierra de Imias: Puntón del Mate.

Pentacalia shaferi (Greenm.) Borhidi comb. nova -- Cuba

Basionym: Senecio shaferi Greenm. Field. Mus. Hist. Nat. 2 (8). 326. 1912. Type: SHAFER 3107. Sierra de Nipe: Punta Gorda.

Pentacalia trichotoma (Greenm.) Borhidi comb. nova -- Cuba

Basionym: Senecio trichotomus Greenm. Field. Mus. Hist. Nat. 2 (8). 326. 1912. Type: SHAFER 3821, Sierra de Nipe; Loma Mensura.

Pentacalia trineura (Griseb.) Borhidi comb. nova -- Cuba

Basionym: Senecio trineurus Griseb. Plant. Wright. Anal. Amer. Acad. 2: 514. 1860. Type CH. WRIGHT 324. Monteverde, Guantánamo.

Lachnorhiza micrantha (Borhidi) Borhidi sp. n.

Basionym: Lachnorhiza piloselloides A. Rich. ssp. micrantha Borhidi Acta Bot. Hung. 29: 214. 1983.

Recently more material were collected in the Cajalbana Hills. The described characteristics turned to be very consequently repeated in the Cajalbana populations, without intermediate forms to L. piloselloides. Therefore I prefer consider this taxon as a good serpentine species, endemic to the mentioned area.

Cupressaceae

According to John SILBA (Phytologia 56/5: 339--341) Juniperus lucayana Britt. is identical with J. barbadensis L. and the local species are to be considered as varieties. Accepting the starting concept of SILBA and maintaining the species concept of BORHIDI (1983: 182; 1979: 32) the following combinations are suggested:

Juniperus barbadensis L. ssp. urbaniana (Pilger & Ekm.) Borhidi comb. et stat. nov.

Basionym: Juniperus urbaniana Pilger & Ekm. in Ark. Bot. 20A 15: 9. 1926. — Hispaniola.

Syn.: J. barbadensis L. var. urbaniana Silba Phytologia 56/5: 340.
Juniperus barbadensis L. ssp. saxicola (Britt. & Wils.) Borhidi comb. nova et stat. novus

Basionym: Juniperus saxicola Britt. et Wils. Bull. Torr. Bot. Club 50: 35. 1923. — Cuba.

REFERENCES

- Alain, H. Liogier (1962): Flora de Cuba. Vol. 5. Caracas, 362 pp.
- Bisse, J. (1985): El género Mosiera Small (Myrtaceae-Myrtoideae) en Cuba, I. Rev. Jard. Bot. Nac. Cub. 6 (3): 3-6.
- Borhidi, A. (1983): New names and new species in the flora of Cuba and Antilles, III. Acta Bot. Hung. 29: 181-215.
- Borhidi, A., Fernandez, M. Z. (1989): El género Exostema L.C. Rich. (Rubiaceae) en Cuba. Acta Bot. Hung. 35: 287-307.
- Fernandez, M. Z., Borhidi, A. (1985): Studies in Rondeletieae, VIII. Nuevos taxa del género Rondeletia en Cuba. Acta Bot. Hung. 31: 147-172.
- Moncada, M. (1989). Reporte del género Commiphora Jacq. (Burseraceae) para Cuba. Rev. Jard. Bot. Nac. Cuba 10: 3-9.
- Nicolson, D. H. (1979): Neolaugeria, a new name for Terebraria (Rubiaceae) of the West Indies. Brittonia 31: 119-124.
- Nordenstam, B. (1978): Taxonomic studies in the Senecioneae (Compositae). Opera Bot. 44: 1-83.
- Proctor, G. R. (1982): More additions to the Flora of Jamaica. J. Arn. Arb. 63: 199-315.
- Robinson, H., Cuatrecasas, J. (1978): A review of the Central American species of Pentacalia (Asteraceae: Senecioneae). Phytologia 40: 37-50.
- Silba, J. (1989): A new combination in Juniperus barbadensis L. (Cupressaceae). Phytologia 56/5: 339-341.
- Urban, I. (1926): Plantae Haitienses novae vel rariores II. Ark. Bot. 20A (5): 1-65.
- Urban, I. (1931): Plantae Haitienses novae vel arariores IX. Ark. Bot. 23A (11): 1-95.

A NEW ERYTHROXYLUM SPECIES IN CUBA

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A new Erythroxylum species was detected in the Maisi Plain, easternmost part of Cuba, living in wind-shaven littoral thicket. It was collected several times by different authors in sterile state and misidentified. An abundant fertile collection made by the first author permitted the recognition and description of a new species as E. armatum.

On the Maisi Plain, the western coast of the Windward Passage, in the easternmost point of Cuba, a particularly interesting wind-blown littoral and semilittoral thicket is developed, partly with scattered emergent tall individuals of the Coccothrinax alexandri palm. Because of the strong wind effect and salt-spray the thicket is composed partly by evergreen, leafless, and deciduous shrubs. This interesting plant community was described by several authors (MARIE-VICTORIN and LEÓN, LEÓN and SEIFRIZ) and also phytosociologically analyzed (BORHIDI *et al.* 1979, BORHIDI 1991). In the composition of the thicket several Erythroxylum species are represented, some of them playing an important role as frequent or locally dominant species, like E. rotundifolium, E. spinescens and a further unknown species mentioned under different names by the different authors, e.g. E. brevipes by LEÓN and SEIFRIZ, E. longipes by MARIE-VICTORIN and LEÓN, and E. pedicellare by BORHIDI. The misunderstanding of this species radicated mostly on the fact, that the shrub is seasonally deciduous — mostly in the winter period, when many botanists visited the region — sometimes even in the rainy season as well, because of the strong wind effect. At last the first author succeeded to collect complete materials of this very interesting species, taxonomically related to E. spinescens, an other littoral spiny species of this genus. The new species differs from E. spinescens by its prostrate habit and longer tertiary branches, short, triangular, usually bilobate stipules densely grouping at the end of the branches and by having broad obovate to

suborbicular smaller leaves with dense emergent reticulum of lateral veins and without areole beneath. The flowers have acute calyx lobes and the petals are 2 times as long as in the E. spinescens A. Rich.

The description of the new species is the following:

Erythroxylum armatum Oviedo et Borhidi spec. nova

Frutex ramosissimus prostratus, ramis spinescentibus, rami hornotini grisacei, lenticellis brevibus vel orbicularibus dispositis, juveniles pur-purascentes et longitudinaliter striati vel sulcati. Ramuli laterales cum tertiariis 0.5-1 cm longis, in foliis 2 basi densissime stipulatis terminantes. Stipulae triangulares, breves, postremo bilobatae, 1-2 mm longae. Folia late obovata vel suborbicularia, antice rotundata vel excisa o emarginata rariter apice mucronata, basi breviter attenuata, 0.5-1.5 cm longa et lata; nervo medio utrinque prominulo, ad medium laminae conspicue magis prominenti, apicem versus evanescenti, laterales utroque latere 6-8, prominulis et dense reticulatis subtus non areolatis. Petiolum tenue et brevissimum, ad 1 mm longum, lamina glabra, supra viridis, subtus flavescens, chartacea.

Flores laterales, plerumque solitarii, basi rosetta stipularum circumdati. Pedunculus angulatus, glaber, 1-3 mm longus. Calyx 5-lobatus, basi connatus, lobi oblongo-ovati, apice acuti, tubo 3-4-plo longiores. Petala 5, calyce 2-3-plo longiora, obtusa, 2 mm longa; stamina 10, filamenta basi connata, tubus filamentorum lobis calycinis brevior. Fructus oblongatus, non pleno maturus 6-7 mm longus, 1-1.5 mm latus; semen oblongo-ellipticum, 5-6.5 mm longum, 4-costatum.

Holotypus: HAC 39163; Cuba; Prov. Guantanamo, Maisi; El Canto, al este de la Cueva del Agua; en matorral xeromorfo costero y subcostero, sobre suelo caliza, diente de perro. Leg.: RAMONA OVIEDO et al. Isotypus: BP.

Obs.: Erythroxylum spinescens A. Rich. affinis, quae a specie nostra habitu alta, ramis dense lenticellatis, lenticellis oblongis praeditis, ramulis tertiariis brevissimis, stipulis dense copertis, stipulis oblongo-ovatis, longe attenuatis, foliis obovatis 1.5-3 cm longis, basi longe attenuatis, subtus obscure areolatis, nervo medio in apice terminato, nervis lateralibus reticulo laxiore, minusque conspicuo, floribus lobis calycinis acutis, petalis 2-plo longioribus differt.

Specimina examinata: Prov. Guantanamo: El Canto, Maisi, Baracoa, Leg.: R. OVIEDO, HAC 33736; -- Maisi entre Cueva del Agua y El Canto, Punta Maisi; leg.: GENES, GUTIERREZ et OVIEDO, HAJB 59211; Prov. Pinar del Rio: Cabo

Corrientes, costas de las Peñas, 20 km al NE de Jaimanitas. Leg.: ARECES, BERAZAIN et DIETRICH, HAJB 34357, 28286 HAC.

The species grows in extreme dry calcareous rocky thickets, probably endemic to Cuba.

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REFERENCES

- Borhidi, A. 1976: Plantas nuevas en Cuba, V. — Acta Bot. Acad. Sci. Hung. 22: 295—320.
- Borhidi, A. 1991: Phytogeography and Vegetation Ecology of Cuba. Akad. Kiadó, Budapest, 858 pp.
- Borhidi, A., Muñiz, O., Del Risco, E. 1979: Clasificación fitocenológica de la vegetación de Cuba. — Acta Bot. Acad. Sci. Hung. 25: 263—301.
- León, H. Sauget, 1942: Vegetación de las terrazas de Maisi. — Rev. Soc. Geogr. Cuba 15/2: 33—49.
- León, H. Sauget, Alain, H. Liogier 1952: Flora de Cuba II. La Habana, 456 pp.
- Marie-Victorin, F., León, H. Sauget 1942, 1944: Itinéraires Botaniques dans l'Ile de Cuba I—II. — Contr. Inst. Bot. Univ. Montreal, No. 41. 496 pp., No. 50. 410 pp.
- Seifríz, W. 1940: Die Pflanzengeographie von Cuba. — Bot. Jahrb. 70: 441—462.

A REVIEW OF BRAZILIAN REPRESENTATIVES OF THE CHRYSOTRICHIA SPECIES GROUP
IN THE GENUS TABEBUIA GOMES EX DC. (BIGNONIACEAE)

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References to early records of Brazilian yellow-flowered species of Tabebuia are given. A key is provided for the 11 Brazilian species of Tabebuia, species group chrysotricha, together with a survey of the keyed taxa and selected synonyms. Another survey refers to the more recent authors who have dealt with the individual taxa and their vernacular names. Of these taxa Tabebuia chrysotricha although typified formerly with reference to Martius' herbarium specimen from Espírito Santo, Brazil, is becoming subject to a new typification. LUSCHNATH's specimen of Tecoma chrysotricha from Corcovado, Brazil, in deposit at the Jardin Botanique National de Belgique (BR) has been identified and designated as the correct lectotype of T. chrysotricha.

Introduction

Among the non-climbing Bignoniaceae of the New World tropics the genus Tabebuia, or Trumpet Tree, comprises species highly prized as ornamentals. Because of their conspicuous beauty and widespread occurrence within their natural ranges, Tabebuia species are believed to have been familiar to highly-developed cultures such as the Incas, Aztecs, and Mayas (CROMWELL 1978).

One of the most characteristic species groups in the genus Tabebuia, that of the Golden Trumpet Tree (T. chrysotricha) includes a number of South as well as Central American yellow-flowered species. Of these T. chrysotricha has from now the purely optional privilege to denote our species group. The character termed "capsula pilosa" by SAMPAIO (1935) is found in the following species of the chrysotricha group: chrysotricha, catarinensis, and ochracea; it is expected to occur in incana, too. Tabebuia ochracea being a typical component of the Brazilian cerrado, the fruit definition of cerrado species of Tabebuia (5 unnamed spp.) as given by RATTER (1986: 56) should be amended to "pericarp not tuberculate, glabrous or tomentose" (in lieu of "pericarp smooth").

First records of Brazilian yellow-flowered bignonies (*Bignonia*) can be found in a book of travels describing the expedition of the Prince of WIED-NEUWIED (1820—21) to Brazil:

WIED-NEUWIED, 1:80 — reference to two vernacular names, viz. Ipé-amarello and Ipé-Tabacco;

id., 1:91 — reference to "hohe mit goldgelben Blumen überschüttete Trompetenbäume (*Bignonia*)";

id., 1:126 — reference to "Trompetenbäume (*Bignonia*), Ipé-amarello genannt, mit grossen gelben Blumen überdeckt, die vor dem Laum ausbrechen".

All the above-mentioned passages refer obviously to yellow-flowered *tabebuias* (for extant herbarium sheets see TOLEDO 1952).

The next authors to be familiar with Brazilian yellow-flowered bignonies were MARTIUS (1824:16: "... mehrere Bignonien ihre goldgelben Blüten entfalten"; p. 16 sub linea: "*Bignonia chrysantha* u.a.") and SAINT-HILAIRE (1824: XXX: "des Bignonées à fleurs jaunes"). The former author (MARTIUS 1828, see also SPIX and MARTIUS 1823) as well as STEAINS (1888), mentioned again *Bignonia chrysantha* Willd. (1800) (Páo d'Arco, vernacular name) to be the main representative of the yellow-flowered species in certain parts of Brazil. In 1837 MARTIUS in his *Herbarium Florae Brasiliensis* let stand Vellozo's *Bignonia longiflora* under *Iecoma* as *Iecoma speciosa* DC. in litt.: "Arbor 50. ped. alt., floret eo tempore, quo folia non habet. In M. Corcovado, prope Sebastianopoli /i.e. Rio de Janeiro, GB/". However, TOLEDO (1952) has shown that the synonymy proposed by MARTIUS was incorrect and to be replaced by the following one. *Iecoma speciosa* DC. as proved indistinguishable from *I. araliacea* Cham. becomes a synonym of *Tabebuia serratifolia* (Vahl) Nicholson.

SALDANHA DA GAMA (1868) compiled many vernacular names of Brazilian plants comprising also those for some species of *Iecoma* (now largely *Tabebuia*): Ipê tabaco, Páo d'arco amarello, and Páo d'arco. Unfortunately, the botanical names given for these as equivalents appear all to be false or imperfect. Ipê tabaco should now refer to *I. chrysotricha*, Páo d'arco amarello to *I. serratifolia*, and Páo d'arco (Black Poui, Poui noir) either to *I. chrysantha* or (in Brazilian specimens) to *I. ochracea*. Similarly, the Yellow Poui (Poui jaune) is nothing else than *I. serratifolia* (and not *I. spectabilis* = *Cydista equinoctialis*, a liana with corolla white or lavender!).

Key to the Brazilian yellow-flowered *Tabebuia* species:

- 1 a Leaflets green above, pubescent or glabrescent, not tomentose (*chrysotricha*, *incana*, *umbellata*, *ochracea*, *vellosi*, *catarinensis*, *bureauvii*, *serratifolia*) 2
- b Leaflets white or yellowish white above, with a dense tomentum composed of branched hairs (*alba*, *pulcherrima* *caraiba*) 9
- 2 a Leaflets densely tomentose beneath 3
- b Leaflets green beneath, pubescent or glabrescent 4
- 3 a Leaflets with golden-brown fuzz beneath; calyx and capsule clothed with woolly and intergrown golden-brown hairs; lobes of calyx erect, not reflexed; leaves with 5 leaflets to 10 cm long; semievergreen lowland tree to 8 m or more (natural range: Pernambuco, Paraná, Santa Catarina; widely cultivated) *T. chrysotricha* and *T. chrysotricha* var. *obtusata*
- b Leaflets with canescent, appressed-stellate tomentum beneath; calyx densely stellate rufescent, capsule unknown; leaves with 5 leaflets to 12 cm long; 25 to 30 m tall canopy tree of Central Amazonia .. *T. incana*
- 4 a Calyx golden woolly-pubescent with many long (to 7 mm) simple trichomes in addition to the short-stellate tomentum, its lobes erect, not reflexed; blooming on bare branches before the new foliage; inflorescence a contracted "terminal panicle" (thyse), the pedicels and peduncle obsolete; corolla of drying flowers with venation becoming obscure in the lobes, the lobes appearing yellow and distinctly different from darker-drying corolla tube (GENTRY 1973); leaves palmately 5-foliate, rarely 7-foliate, entire to serrate, densely pubescent beneath; capsule abundantly golden woolly pubescent: in tropical dry forest
 — cerrado *T. ochracea* and *T. ochracea* subsp. *heteropoda*
- b Calyx otherwise 5
- 5 a Capsule densely reddish-brown tomentose with mostly barbate trichomas to 1 mm long; calyx pilose with reddish mostly barbate trichomes; highland shrub to 3 m (Santa Catarina, Paraná) *T. catarinensis*
- b Capsule smooth-surfaced, glabrous 6
- 6 a Calyx lepidote, with very short, brownish, branched hairs; leaves palmately 5- to 7-foliate or (*fosteri*) only 3- to 5-foliate; leaflets basally entire, sometimes dentate at the tips; tree 15 to 20 m tall, in North American cultivation (*fosteri*) smaller (only to 5 m); blooming on bare branches before the new foliage; natural distribution restricted to pluvial forests of the Atlantic shore (Minas Gerais, Rio de Janeiro,

- Guanabara, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul)
 I. umbellata and I. umbellata var. lanceolata
 b Calyx otherwise again 7
 7 a Calyx very shortly stellate-rufescent; leaflets conspicuously serrate;
 tree to 12 m, endemic to the vicinity of Rio de Janeiro .. I. bureauvii
 b Calyx slightly hairy 8
 8 a Ovary 5 to 7 mm long; corolla 8 to 10 (12) cm long; capsule 30 to 40 cm
 long and 1.5 to 2 cm wide; 5 to 7 leaflets, sharply and closely ser-
 rate; flowers appear prior to the new foliage and coexist even with
 mature leaves I. vellosi
 b Ovary 2 to 4 mm long; corolla 6 to 8 (10) cm long; capsule 20 to 35 cm
 long and 2.5 to 3.5 cm wide; 4 to 5 leaflets, serrate or crenate-ser-
 rate, rarely entire; blooming on bare branches before the new foliage;
 tree 5 to 15 m, occasionally 20 to 25 m tall, copious in pluvial for-
 ests, especially Amazonia I. serratifolia
 9 a Mature leaves large, 5- to 7-foliolate; mature leaflets serrate, often
 longer than 10 cm; blooming with multiflorous thyrses; capsule 1.5 to
 2.5 wide; seeds 7 to 8 mm (7 to 9 mm according to SANDWITH and HUNT
 1974: 31) "long" (= broad, cf. PACLT 1952); highland tree to 20 m, more
 rarely to 30 m tall (Minas Gerais, Rio de Janeiro, Paraná, Santa Cata-
 rina, Rio Grande do Sul) I. alba
 b Mature leaves relatively small, mostly less than 10 cm long 10
 10 a Blooming with rather contracted multiflorous thyrses: capsule less than
 1.5 cm wide; seeds less than 5 mm "long" (= broad, cf. PACLT 1952);
 tree usually 5 to 10 m tall (Santa Catarina, Rio Grande do Sul)
 I. pulcherrima
 b Blooming with loose pluriflorous thyrses; capsule 2 to 2.5 cm wide;
 tree 6 to 8 m tall, in tropical dry forest -- cerrado I. caraiba

Survey of the keyed taxa and selected synonyms

Tabebuia chrysotricha (Mart. ex DC.) Standley

Syn.: Bignonia chrysantha Mart. nec. Jacq.

Tecoma flavescens Mart. ex DC. excl.syn.

? Catalpa hirsuta Sprengel[§] (cf. SPRENGEL 1825 et PACLT 1952)

I. chrysotricha var. obtusata (DC.) Toledo

[§]The type for the taxon is SPRENGEL's description as no trace has been found of the specimen upon which SPRENGEL based his name and description. SPRENGEL himself did not mention any collector's name!

- T. incana A.Gentry
T. ochracea (Cham.) Standley
 Syn.: Tecoma ochracea Cham.
Tecoma hypodictyon DC.
T. ochracea subsp. heteropoda (DC.) A.Gentry
T. catarinensis A.Gentry
T. umbellata (Sonder) Sandwith
T. umbellata var. lanceolata (Bur. et K.Schum.) Toledo
T. bureauvii Sandwith
 Syn.: Tecoma dentata Bur. et K.Schum. nec Miers (pro Tabebuia)
T. vellosi Toledo
 Syn.: Bignonia longiflora Vellozo^{§§}
 non Tabebuia longiflora (Griseb.) Greerim.
T. serratifolia (Vahl.) Nichols.
 Syn.: Bignonia flavescens Vellozo (cf. GENTRY 1975)
Tecoma speciosa DC.
T. alba (Cham.) Sandwith
 Syn.: Tecoma alba Cham.
T. pulcherrima Sandwith
T. caraiba (Mart.) Bur.

Survey of species and infraspecific taxa mentioned by the more recent authors (cp. References)

- Tabebuia sp., group chrysotricha: HOEHNE 1939 (Ipê Amarelo, p. 271).
Tabebuia chrysotricha: TRAVASSOS 1956 (Ipê-tabaco); FABRIS 1959; HUECK 1966 (Ipê amarello); SANDWITH et HUNT 1974 (Ipê-do-morro, Ipê-amarelo, Ipê-tabaco, Aipé, Ipê, Pau-d'arco-amarelo); SIMERLY 1974; CARVALHO et al. 1976 (Ipê-amarelo); SANTOS 1976 (Ipê tabaco); BUZZI ZUNDIR 1976; CROMWELL 1978; DUARTE 1979 (Ipê-tabaco, Ipê caboclo); SANTOS 1979 (Ipê tabaco); ANONYMUS 1981; NICOLETTI et al. 1984 (Ipê coscovo from Porto Alegre, RS); ALCALAY et al. 1985 (Ipê amarelo); GRAF 1986; FIGLIOLIA 1988 (Ipê-amarelo-añao).
Tabebuia chrysotricha var. obtusata: TOLEDO 1952 (Ipê merim, Ipê mulato, Ipê).
Tabebuia incana: GENTRY 1978 (Pau d'arco amarelo from Belterra, PA).
Tabebuia ochracea: HUECK 1966 (Ipê pardo); HOGREBE 1979 (Lapacho amarillo, Tayisaiyu); GIBBS et al. 1983; GOTTSBERGER et SILBERBAUER-GOTTSBERGER 1983; SILBERBAUER-GOTTSBERGER et EITEN 1983; FERREIRA et HENNEN 1986; CESAR et al. 1988; MANTOVANI et MARTINS 1988; PAGANO et al. 1989 a-b.
Tabebuia ochracea subsp. heteropoda: PRANCE et SCHALLER 1982.
Tabebuia catarinensis: GENTRY 1977.
Tabebuia umbellata: HUECK 1966; SANDWITH et HUNT 1974 (Ipê-amarelo, Ipê-da-várzea, Ipê-da-vargem, Ipê); DEMONTE 1985 (from Petrópolis 1984, RJ); GRAF 1986.
Tabebuia umbellata var. lanceolata: TOLEDO 1952 (Ipê do brejo).
Tabebuia bureauvii: GENTRY 1974.
Tabebuia vellosi: TOLEDO 1952 (Ipê do grande, Ipê amarelo, Ipê); Sociedade Brasileira de Floricultura 1954; HANDRO 1954 (Ipê-rei); TRAVASSOS 1956 (Ipê-amarelo); PACHECO 1972 (Ipê amarelo from GB); DUARTE 1979 (Ipê-amarelo); RIZZINI 1986; FIGLIOLIA et al. 1988 (Ipê-amarelo).
Tabebuia serratifolia: MIYASAKI et CÂNDIDO 1978 (Ipê-amarelo); FREITAS et al. 1979 (Ipê amarelo); MUCHOVEJ et FERREIRA 1981 (yellow Ipê from Viçosa, MG); ? BIANCO et al. 1982 a-c (Ipê amarelo); NICOLETTI et al. 1984 (Pau d'arco amarelo from Belém, PA); FERREIRA et HENNEN 1986 (yellow Ipê from Viçosa, MG); RIZZINI 1986 (Pau-d'arco-amarelo), Piúva-amarela, Ipê-ovo-de-macuco, Opa); FERREIRA et MUCHOVEJ 1987 (yellow Ipê from Belém, PA; ? Ipê-Ovo-de-Macuco from Linhares, ES).

^{§§}Vernacular names given by VELLOZO: Ipéuva, Ipê-do-campo.

Tabebuia alba: TRAVASSOS 1956 (Ipê-mandioca); SANDWITH et HUNT 1974 (Ipê-da-serra, Ipê-mandio-ca, Ipê-branco etc.); GENTRY 1977; NICOLETTI et al. 1984 (Ipê branco from Belo Hori-zonte, MG); FERREIRA et HENNEN 1986.

Tabebuia pulcherrima: SANDWITH et HUNT 1974 (Ipê-da-praia etc.); NICOLETTI et al. 1984 (Ipê da praia from Pôrto Alegre, RS).

Tabebuia caraiba: FABRIS 1959; RIBEIRO et al. 1979 (with a very complete list of vernacular names); PRANCE et SCHALLER 1982 (Paratudo); GIBBS et al. 1983; PERES et BERTI 1985; MANTOVANI et MARTINS 1988.

Correct lectotype for *Tecoma chrysotricha* Mart. ex DC.

Consistent with SANDWITH and HUNT (1974), *Tabebuia chrysotricha* (Mart. ex DC.) Standley has been typified with reference to the herbarium specimen "Brasil, Espírito Santo, Martius (M)".

When ALPHONSE DE CANDOLLE described *Tecoma chrysotricha* in 1845, he mentioned four specimens representing formally syntypes of that species:

- in Brasilia, LUND;
- ad Sanctam Theresam juxta Rio de Janeiro, GUILLEM.;
- Corcovado, LUSCHNATH;
- in campestribus prov. Sancti Spiritus, MARTIUS —

Of available herbarium sheets the specimens collected by LUND and LUSCHNATH respectively are preserved at BR, that by MARTIUS at M.

Although the species has already been typified,^{§§§} we propose herewith to revise that typification. One herbarium sheet, that of "Brasilia, LUND" with a determinavit written possibly by DC. (fide A. LAWALRÉE) would not be appropriate for lectotype selection at all.

On the other hand, the specimen collected 1817 by MARTIUS himself and preserved at M consists of vegetative parts only of what is labelled *Tecoma ochracea* Cham. and *T. obtusata* DC. (Kew negative 5951). Indeed, this herbarium sheet is deposited sub *Tecoma chrysotricha* Martius but the original label as well as given locality "in locis campestribus prov. Sancti Spiritus /Espírito Santo/" suggest elimination of that material from the syntypes of the typical *T. chrysotricha*.

According to the current edition of The International Code of Botanical Nomenclature (Regnum Vegetabile, vol. 118, 1988, Art. 8.1) the author who first designates a lectotype or a neotype must be followed, but his choice is superseded if (a) the holotype or, in the case of a neotype, any of the original material is rediscovered; it may also be superseded if (b) it can be shown that it is in serious conflict with the protologue and another element is available which is not in conflict with the protologue, or (c) that it was based on a largely mechanical method of selection, or (d) that it is contrary to Art. 9.2.

There are just the items (b) and (c) of Art. 8.1 that apply to the case of *T. chryso-tricha*. In harmony with these, the first lectotypification of that species is superseded.

The herbarium sheet "Corcovado, LUSCHNATH" in deposit at the Jardin Botanique National de Belgique (BR) includes not only vegetative parts of *Tabebuia chrysotricha*, but also hirsute capsules, and is herewith designated as the appropriate lectotype. The locality, M. Corcovado (in the vicinity of Rio de Janeiro, GB) proves to fall into the actual ranges of at least three yellow-flowered species of *Tabebuia*: *T. bureauvii* (endemic ?, natural range, closely related to *T. umbellata*), *T. chrysotricha*, and, according to Martius (1837), "*Tecoma speciosa* DC. in litt.", the last being almost certainly not the true *T. speciosa* DC. = *Tabebuia serratifolia*, but rather the traditional national tree of Brazil ("flor nacional"), *Tabebuia vellosi*.

^{§§§}Lectotype of *T. chrysotricha* Mart. ex DC. selected mechanically in consideration of the name MARTIUS, one of the syntype collectors and at the same time the nominator of the taxon.

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REFERENCES

- Alcalay, Nara, Irigón, Amaral Dóris Maria, Guadalupe, Antonio Margô (1985): Descrição de plantas de essências florestais do Rio Grande do Sul. Roesslêria 7: 139-156.
- Bianco, A., Passacantilli, P., Nicoletti, M., Alves de Lima, R. (1982a): Iridoids in equatorial and tropical flora. III. Isolation and partial synthesis of 6-epiaucubin, a new glucosidic iridoid. Tetrahedron 38: 359-362.
- Bianco, A., Passacantilli, P., Nicoletti, M., Alves de Lima, R. (1982b): Iridoids in equatorial and tropical flora. Part 4. Isolation of amere loside. Planta medica 46: 33-37.
- Bianco, A., Passacantilli, P., Nicoletti, M., Alves de Lima, R. (1982c): Iridoids in equatorial and tropical flora. V. A new glucosidic irridoid from Tecoma chrysantha Jacq. Gazz. Chim. Ital. 112: 227-229.
- Borhidi, A. (1980): Notas sobre Bignoniaceas Cubanas, I. Acta Bot. Hung. 26: 15-24.
- Buzzi Zundir, J. (1976): Sobre a biologia de Dorynota pugionata (Germar 1824) (Coleoptera, Chrysomelidae). Dusenja 9: 41-46.
- Carvalho, N. M., Goes, M., Aguiar, I. B., Fernandes, P. D. (1976): Armazenamento de sementes de ipê-amarelo (Tabebuia chrysotricha). Científica (Jaboticabal) 4: 315-319.
- Cesar, O., Pagano, S. N., Leitão Filho, Hermógenes F., Monteiro, R., Silva, Osvaldo Aulino de, Marinis, Giorgio de, Shepherd, G. J. (1988): Estrutura fitossociológica do estrato arbóreo de uma área de vegetação de cerrado no município de Corumbataí (estado de São Paulo). Naturalia (São Paulo) 13: 91-101.
- Cromwell, G. (1978): Tabebuia, the Trumpet Tree. Garden (LASCA edition) 22 (4): (L) 5-7. /LASCA = Los Angeles State and Conuntry Arboretum./
- Oemonte, Etienne, Rosália and Yvonne (1985): For Love of Nature. Brazilian flora and fauna in watercolor by ... Hunt. Inst. for Bot. Docum. Pittsburgh; Wave Hill, Bronx, N. Y.
- Duarte, Apparicio Pereira (1979): Contribuição ao conhecimento da germinação das sementes nas essências mais usuais. Rodriguésia 31 (48): 59-68.
- Fabris, H. A. (1959): Bignoniáceas. In: Las plantas cultivadas en la República Argentina 10 (173): 1-57. Inst. de Bot. Agríc. C.N.I.A., Buenos Aires.
- Ferreira, F. A., Hennen, J. F. (1986): The life cycle, pathology, and taxonomy of the rust, Prospodium bicolor sp. nov., on yellow ipê, Tabebuia serratifolia, in Brazil. Mycologia 78: 795-803.
- Ferreira, F. A., Muchovej, J. J. (1987): Asteromidium Tabebuiae sp. nov. from Brazil. Mycotaxon 30: 97-100.
- Figliolia, Márcia Balistiero (1988): Conservação de sementes de essências florestais. Bol. técn. Inst. florest. (São Paulo) 42: 1-18.
- Figliolia, Márcia Balistiero, Silva, Antonio da, Jardim, Denise Calil Pereira, Iwane, Maria Shizue Shin-Ike (1988): Viabilidade de sementes liofilizadas de essências florestais nativas. Silvicultura em São Paulo 20/22: 47-55.
- Freitas, S. C., Cândido, I. F., Condé, A. R., Ara, T. (1979): Determinação de equilíbrio higroscópico e viabilidade de sementes de ipê amarelo (Tabebuia serratifolia /Vahl/ Nichols.) armazenadas em diferentes umidades relativas. Rev. Árvore (Viçosa) 3: 135-144.

- Gentry, A. H. (1973): Flora of Panama, Part IX, Family 172. Bignoniaceae. Ann. Missouri Bot. Gard. 60: 781—977.
- Gentry, A. H. (1974): Studies in Bignoniaceae 12: New or noteworthy species of South American Bignoniaceae. Ann. Missouri Bot. Gard. 61: 872—885.
- Gentry, A. H. (1975): Identification of Vellozo's Bignoniaceae. Taxon 24: 337—344.
- Gentry, A.H. (1977): Studies in Bignoniaceae 25: New species and combinations in South American Bignoniaceae. Ann. Missouri Bot. Gard. 64: 311—319.
- Gentry, A. H. (1978): Studies in Bignoniaceae 31: New species and combinations from Amazonian Peru and Brazil. Ann. Missouri Bot. Gard. 65: 725—735.
- Gibbs, P. E., Leitão Filho, H. de F., Shephard, G. (1983): Floristic composition and community structure in an area of cerrado in SE Brazil. Flora 173: 433—449.
- Gottsberger, G., Silberbauer-Gottsberger, I. (1983): Dispersal and distribution in the cerrado vegetation of Brazil. Sonderbd. Naturwiss. Ver. Hamburg 7: 315—352.
- Graf, A. B. (1986): Tropica. Color Cyclopaedia of Exotic Plants and Trees. Ed. 3. Roehrs Company — Publishers, East Rutherford, New Jersey.
- Handro, O. (1954): O Ipê, uma jóia da flora brasileira. Flôres do Brasil (São Paulo) 1 (1): 8—10.
- Hoehne, F. C. (1939): Plantas e substancias vegetais tóxicas e medicinais. Dept. de Bot. do Estado, São Paulo.
- Hogrebe, H. (1978): Boden und Vegetation im Raum de Iguazu-Wasserfälle am Dreiländereck Brasilien, Argentinien, Paraguay. Mitt. Dtsch. Dendrol. Ges. 70: 229—232.
- Hueck, K. (1966): Die Wälder Südamerikas. Ökologie, Zusammensetzung und wirtschaftliche Bedeutung. Fischer-Verl., Stuttgart.
- Mantovani, W., Martins, F. R. (1988): Variações fenológicas das espécies do cerrado da Reserva Biológica de Moji Guaçu, Estado de São Paulo. Rev. Brasil. Bot. 11: 101—112.
- Martius, C. F. P. von (1824): Die Physiognomie des Pflanzenreiches in Brasilien. — Eine Rede gelegen zur Feier der fünfundzwanzigjährigen glorreichen Regierung Seiner Majestät des Königs am 14. Februar 1824 gehaltenen ausserordentlichen festlichen Sitzung der Königl. Baier. Akad. der Wiss., München.
- Martius, C. F. P. von, Spix, J. B. von, Martius, C. F. P. von (1828): Reise in Brasilien, vol. 2 (post mortem Spixii). München.
- Martius, C. F. P. de (1837): Herbarium florae brasiliensis. Beibl. Allg. Bot. Z. 2: 1—352. Monachii.
- Miyasaki, J. M., Cândido, I. F. (1978): Secagem de sementes de ipê-amarelo (Iecoma serratifolia (Vahl)Don). Seiva (Viçosa) 39 (85): 12—17.
- Muchovej, J. J., Ferreira, F. A. (1981): A new species of Phaeoramularia from Brazil. Mycologia 73: 345—347.
- Nicoletti, M., Serafini, M., Bianco, A., Passacantilli, P. (1984): Recent acquisitions of iridoids in Bignoniaceae. Annali di Bot. (Roma) 42: 81—90.
- Pacheco, J. M. (1972): Contribuição ao estudo botânico da Iabebuia longiflora Greenm.- (Iecoma longiflora, Bignoniaceae). Atlas Soc. Biol. Rio de Janeiro 16 (1): 1—4.
- Paclet, J. (1952): Synopsis of the genus Catalpa (Bignoniaceae) III. Special part. Candollea 13: 241—285.
- Pagano, Sergio Nereu, Cesar, O., Leitão-Filho, Hermógenes de Freitas (1989a): Composição florística do estrato arbustivo-arbóreo da vegetação de cerrado da área de proteção ambiental (APA) de Corumbataí — Estado de São Paulo. Rev. Brasil. Biol. 49: 37—48.

- Pagano, Sergio Nereu, Cesar, Oswaldo, Leitão-Filho, Hermógenes de Freitas (1989b): Estrutura fitossociológica do estrato arbustivo-arbóreo da vegetação de cerrado da área de proteção ambiental (APA) de Corumbataí — Estado de São Paulo. Rev. Brasil. Biol. 49: 49—59.
- Peres, O., Berti, E. (1985): Biologia de Rolepa unimoda (Dognin 1923) (Lepidoptera, Lymantriidae) em folhas de Tabebuia avellanedae Lor. ex Griseb. (Bignoniaceae) e observações sobre inimigos naturais. Anais Esc. super. Agric. Luiz de Queiroz 42 (1): 55—82.
- Prance, G. T., Schaller, G. B. (1982): Preliminary study of some vegetation types of the Pantanal, Mato Grosso, Brazil. Brittonia 34: 228—251.
- Ratter, J. A. (1986): A key to the woody genera of dicotyledons of the Brazilian cerrado. Notes Roy. Bot. Gard. Edinburgh 44: 45—69.
- Ribeiro, Vera Maria Lúcia, Costa, Elenice de Lima, Barroso, Maria Alice Lima (1979): Catálogo de nomes científicos e vulgares de plantas de porte arbóreo ocorrentes no Brasil. Rodriguésia 31 (49): 155—233.
- Rizzini, C. T. (1978): Arvores e madeiras úteis do Brasil. Manual de dendrologia brasileira. Ed. 2. E. Blücher Ltda., São Paulo.
- Saint-Hilaire, A. de (1824/—26/): Histoire des plantes les plus remarquables du Brésil et du Paraguay. A. Belin, Paris.
- Saldanha da Gama, J. de (1868): Synonymia de diversos vegetaes do Brasil feita segundo os dados colhidos no imperio, e na Exposição Universal de Paris, em 1867 por ... Typographia Universal de Paris, Rio de Janeiro.
- Sampaio, A. J. de (1935): Caracteres de menor frequencia nas Bignoniaceae. Arch. Mus. Nac. (Rio de Janeiro) 36: 73—85.
- Sandwith, N. Y., Hunt, D. R. (1974): Bignoniáceas. In: Reitz, R. (ed.): Flora Ilustrada Catarinense I. (Tabebuia, p. 14—40).
- Santos, N. dos (1976): Plantas existentes no Parque Nacional da Tijuca. Brasil Forestal 7 (26): 54—68.
- Santos, Nila dos (1979): Contribuição ao estudo da flora existente no Parque Nacional da Tijuca. Rodriguésia 31 (51): 71—126.
- Silberbauer-Gottsberger, I., Eiten, G. (1983): Fitossociologia de um hectare de cerrado. Brasil Florestal 14 (54): 55—70.
- Simerly, F. (1974): Plants to look for. LASCA Leaves 24: 10—12.
- Sociedade Brasileira de Floricultura (1954): Nossa capa. Ipê em plena floração. Flôres do Brasil (São Paulo) 1 (1): 3, colour illustration on front page of the cover.
- Spix, J. B. von, Martius, C. F. P. von (1923): Reise in Brasilien, vol. I. München.
- Sprengel, C. (1825): (Linnaeus) Systema Vegetabilium, ed. 16, vol. 1. Gottingae.
- Steains, W. J. (1888): An exploration of the Rio Dôce and its northern tributaries (Brazil). Proc. R. Geogr. Soc. 10: 61—79. (Discussion, p. 79—84; map behind p. 116.)
- Toledo, J. F. (1952): Notulae de aliquot plantis brasiliensibus novis vel minus cognitis. Arq. Bot. Estado Saõ Paulo, n.s., in quarto, 3 (1): 27—44.
- Travassos, O. P. (1956): Floração de inverno. Flôres do Brasil (São Paulo) 2: 58—61.
- Wied-Neuwied, Maximilian Prinz zu (1820—21): Reise nach Brasilien in den Jahren 1815 bis 1817. vol. 1 (= 1820), 2 (= 1821). H. L. Brönnner, Frankfurt a. M.
- Anonymus (1981): Pink and gold bouquets in the Los Angeles sky. Sunset Magazine 166 (4): 92—93.

EKMANIOPAPPUS BORHIDI GEN. NOVUM (SENECIONEAE, ASTERACEAE)
IN HISPANIOLA

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Three species has been described within the framework of the genus Herodotia Urb. et Ekm.: H. haitiensis (type of genus), H. mikanioides and H. alainii. The two later closely related species are far distant from the typical species of the genus. Their descriptions do not correspond to the generic criteria of Herodotia, given by URBAN and EKMAN. JIMENEZ amplified the description of Herodotia for including all the three species, but in this case the description became very inconsistent with uncertain generic limits. Authors taking in consideration new generic criteria within the tribe Senecioneae, revised the morphological characters of the taxa and found new anatomical evidences for distinguishing them. Based on these results, Herodotia is suggested to be maintained in the original sense of URBAN and EKMAN for H. haitiensis and to establish the new genus Ekmaniopappus for the two other species, with E. mikanioides (type of genus) and E. alainii.

Introduction

Herodotia Urb. et Ekm. was described (Ark. Bot. 20A 5: 63-64. 1926) based on the specimen collected by EKMAN in Morne Trachant, Massif de la Selle in the Jaragua Peninsula, Haiti, under the collection number H. 1881, and named as Herodotia haitiensis Urb. et Ekm.

The generic description contained the following important diagnostic elements: capitula homogamous, flowers 2-3, hermaphrodite, yellow, inflorescence corymbiform, terminal, involucre bracts 4, achenes paucistriate, leaves opposite, not reticulate.

Later in the same Massif de la Selle, in the Morne Cabaio EKMAN collected his number 1570, which represented another new species. Being this specimen a sterile one, it was insufficient for making a description, but somewhat later EKMAN succeeded to collect a complete material in the Massif de la Selle, under the number H 3123, which permitted to URBAN to describe this new species naming it as Herodotia mikanioides Urb. et Ekm. Ark. Bot. 20A 15: 93 1926. This new species showed a great number of differential

features like leaves with woolly lower surface and spiny margin, terminal and axillar paniculate inflorescences with heterogamous capitula, heads with 5-6 bracts, 8-striate achenes etc.

Including this second species into Herodotia, URBAN had to amplify the original generic description and change "capitula homogama" to "capitula homogama vel heterogama", the "bracteae 4" to "bracteae 4-6", the inflorescencia terminal subcorymbosa" to "inflorescencia terminal subcorymbosa vel terminal y axilar paniculata" etc., but he did not. In consequence the description of Herodotia mikanioides did not satisfy the criteria of the genus Herodotia Urb. and Ekm. Maybe, URBAN waited for intermediate taxa between the two described extreme forms. The drawings of the two species published by URBAN (Plates I and II) show great morphological differences. In the notes of the description URBAN expressed his hesitation about the possibility to consider Herodotia mikanioides as representative of a new genus. He wrote: "The other species of this genus Herodotia haitiensis is far distant by having glabrous surface, leaves acute at the apex, with 4-5 lateral veins, non reticulate connected above, with 4 involucre bracts, homogamous heads 2-3 flowers, anthers obtuse at the base, but perhaps not to be separated on generic level."

The first author of this paper have had a different opinion motivated by four experiences:

1) When he analysed the speciation strategies of the endemic Antillean genera (BORHIDI 1985, 1991), he found that they are consisted of morphologically very similar, closely related allopatric or rarely sympatric species. We don't know any polytypic endemic genus (Schmidtottia, Stevensia, Grisebachianthus, Spaniopappus, Heptanthus etc.), where the component species would be morphologically distant sympatric taxa.

2) This statement has been consolidated by a new founding of H. ALAIN LIOGIER, who collected a third Herodotia species in the Dominican Republic, Cañada del Convento, Constanza, Provincia de La Vega, described by J. JIMENEZ (1977) as Herodotia alainii. This species is obviously an allopatric one and it is very closely related to H. mikanioides by its general habit and morphology, while it is even farther situated taxonomically from the typical species of the genus Herodotia.

3) The generic concept within the tribe Senecioneae has changed very much based on the works of NORDENSTAM (1978), CUATRECASAS and ROBINSON (1977, 1981), who introduced a number of new anatomical criteria for recognizing and separating genera of this tribe.

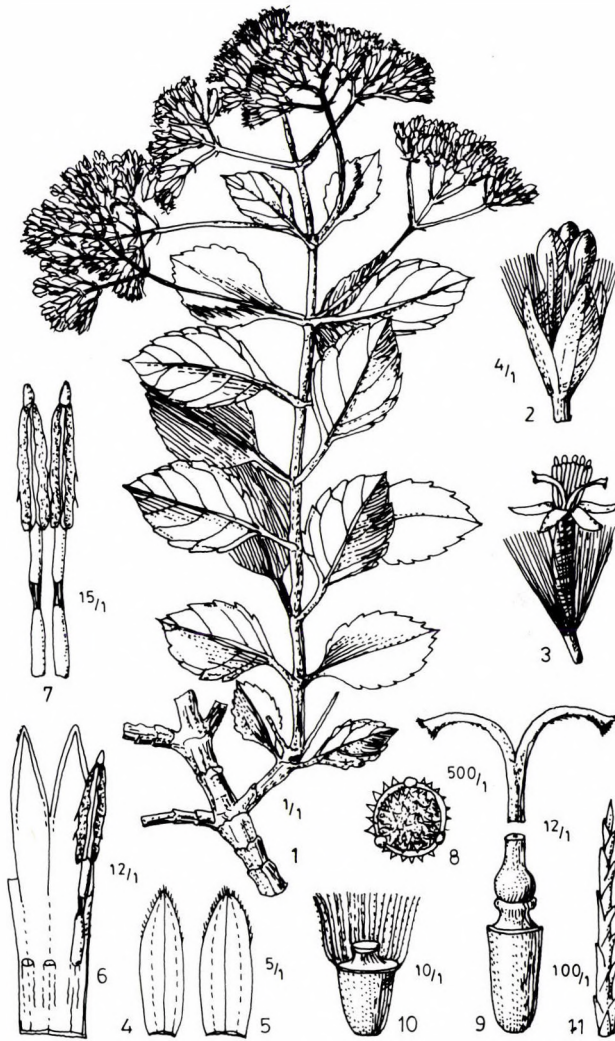


Plate I

Herodotia haitiensis Urb. et Ekm. (original drawings by Urban and Pohl from the Arkiv för Botanik Vol. 20). 1. Flowering plant, 2. Young inflorescence with flower buds, 3. Open flower, 4. and 5. Involucral bracts, 6. Longitudinal section of the corolla with one stamen, 7. Stamens, 8. Pollen grain, 9. Pistil, 10. Young achene, 11. One bristled pappus hair

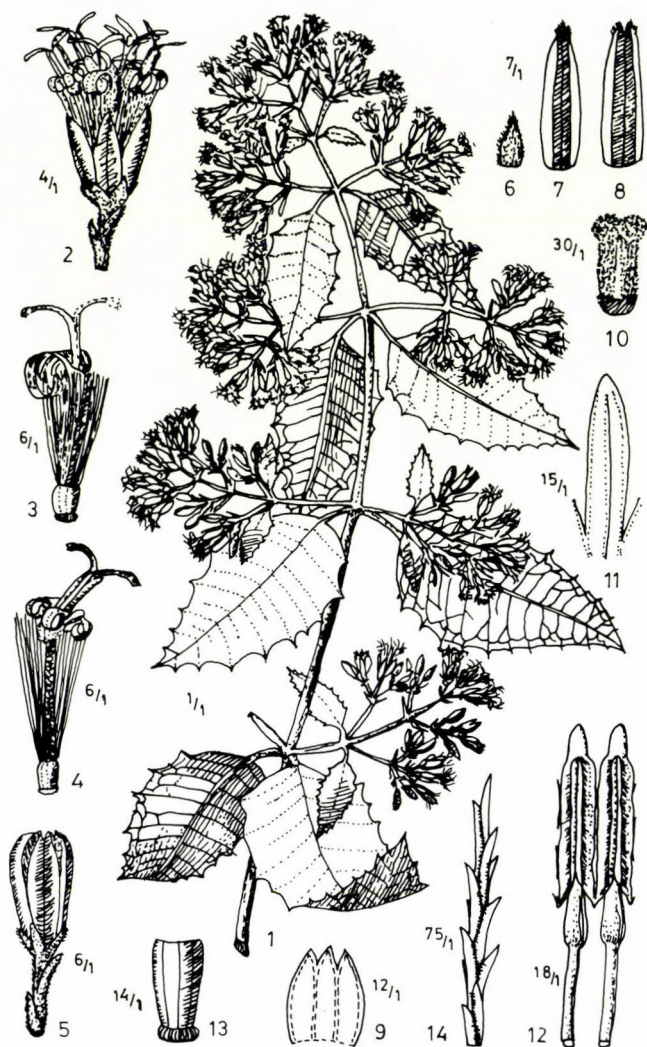


Plate II

Herodotia mikanioides Urb. et Ekm. (original drawings by Urban and Pohl from the Arkiv för Botanik Vol. 20). 1. Flowering plant, 2. Inflorescence with open flowers, 3. Ray flower, 4. Disc flower, 5. Young closed head, 6. Subinvolucral bract, 7. and 8. Involucral bracts, 9. Apical part of the ray flower corolla, 10. Upper part of the style branch from the ray flower, 11. One corolla lobe of a disc flower, 12. Stamens, 13. Ovary with disc, 14. One bristled hair of the pappus

4) JIMENEZ made the above cited amplifications, but the description of the genus Herodotia became very inconsistent with these changes and its generic limits turned to be uncertain.

Results

The authors made a more detailed morphological study (with LM and SEM) of the Herodotia haitiensis and H. mikanioides and found a number of new anatomical features for differentiating the two taxa. Table 1 summarizes the most important differences.

Based upon these studies the separation of the two taxa on generic level is suggested. In consequence, we consider it necessary to re-establish the genus Herodotia Urb. et Ekm. in the original sense, and to establish a new genus for a comprehensive locating of H. mikanioides and H. alainii. This new genus would be named as Ekmaniopappus Borhidi gen. nov. in honour of ERIK LEONARD EKMAN, the first collector of the typical species.

Ekmaniopappus Borhidi gen. nov.

Frutices sarmentosi vel scandentes, domingenses et haitienses. Synflores centice axillares et terminales in paniculam longam compositae. Capitula heterogama radiata, floribus radialibus 2-3 femineis, discoideis 2-3 hermaphroditis. Involucrum cylindraceum erectum, basi bracteolis 3-4 uniseriatis suffultum, squamae involucri 5-7, uniseriatae chartaceae, inaequilatae, 3-5-nervae, margine membranaceae. Receptaculum parvum nudum. Corollae tubulosae floris discoidei, tubus cylindraceus, sub lobi ampliatus, lobi 5, revoluti, corolla florum radialium inferne tubulosa, superne ovata, apice 3-crenulata 3-nervia. Stamina tubo supra medium inserta, filamenta basi articulata apicem versus incrassata. Antherae basi breviter filiformi-caudatae, apice in ligulam oblongam obtusam productae.

Pollinis granula 3-colporata, dense tuberculata, colpus leviter emergens, tuberculae triangulari-oblongae, apice mammiformiter apiculatae.

Stylus bifidus, ramis aequilatis inappendiculatis, supra anguste sulcatis, margine supero obsolete papilloso apice truncatis et brevissime penicillatis.

Achaenia 8-costata, inter costae minute verrucosa, pappus uniseriatus, caducus setae minute spinuligerae.

Folia opposita vel alterna, margine spinuliformi-denticulata subtus araneoso-tomentosa.

Table 1

Main morphological differences between Herodotia and Ekmaniopappus

	<u>Herodotia</u> 1 species	<u>Ekmaniopappus</u> 2 species
Synflorescence	terminal subcorymbose	terminal and axillary forming a large panicle
Heads	homogamous	heterogamous
Flowers	2-3 disc florets hermafrodite, equal in shape and size	5-6 different in shape and size: Ray florets, 2-3 female, Disc florets 2-3 hermaphrodite
Bracteoles	absent	3-4 subinvolucral
Involucral bracts	4, biseriate, opposite, external without, internal with membranous margin	5-6, uniseriate, subequal, all with membranous margin
Adaxial surface	smooth, with stomata	striate with protruding nerves without stomata
tubes	cylindric	shortly broadened below the lobes
Corolla lobes	without nerves	lobes with 1 strong dorsal nerve
Filaments	articulated at the middle not thickening above adnate to the corolla tube under the middle	articulated at the base thickening above adnate to the corolla tube above the middle
Anthers	obtuse at the base, without appendage	with short thread-like appendage
Pollen	3-colporate, big protruding colpi	3-colporate, colpus not protruding
Exine	densely tuberculate with triangular spines	densely tuberculate, with longer mammiform, apiculate spines
Achene	8-sided, glabrous	8-sided ribbed and bristled
Pappus	densely bristled, biseriate with short rectangular or slightly ascendent denticles, permanent	densely bristled, subbiseriate with longer erect denticles, deciduous
Leaves	opposite, glabrous on both surface	opposite and alternate aracnoid-tomentose beneath
Venation	camptodromous, curving towards the apex, not forming loops before the margin	camptodromous, anastomosing forming loops before the margin
Terminal veinlets	get to the margin between the teeth	get to the margin in the teeth, ending in a mucrone
Stoma	anomocytic	cyclocytic

Typus generis: Herodotia mikanioides Urb. et Ekm.

Species huc pertinentes

Ekmaniopappus mikanioides (Urb. et Ekm.) Borhidi comb. nova

Basionym: Herodotia mikanioides Urb. et Ekm. Ark. Bot. 20A; 15: 93--94. 1926.

Typus: EKMAN H 3123!

Ekmaniopappus alainii (J. Jimenez) Borhidi comb. nova

Basionym: Herodotia alainii J. Jimenez Coll. Conf. 2: 15. 1977. Acad. Cien. Rep. Dom.

Typus: ALAIN 22276. Rep. Dom.

Description of the floral parts and leaves based on SEM-study

Herodotia haitiensis Urb. et Ekm.

Bracts

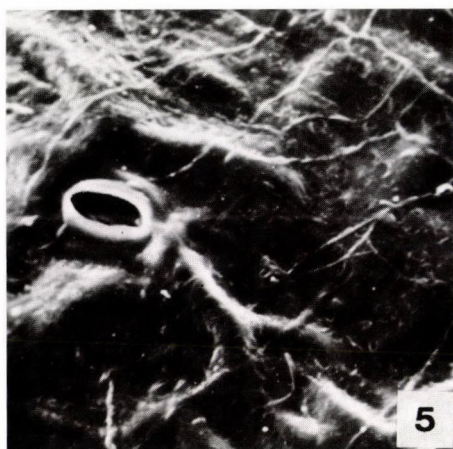
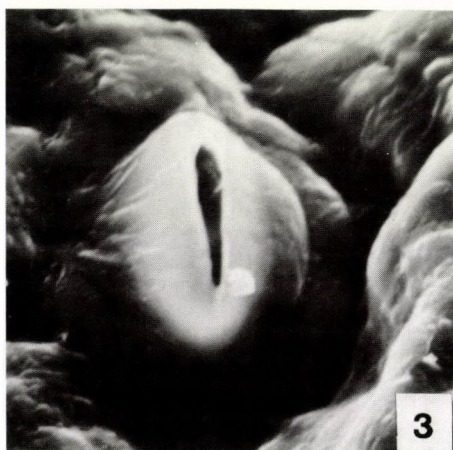
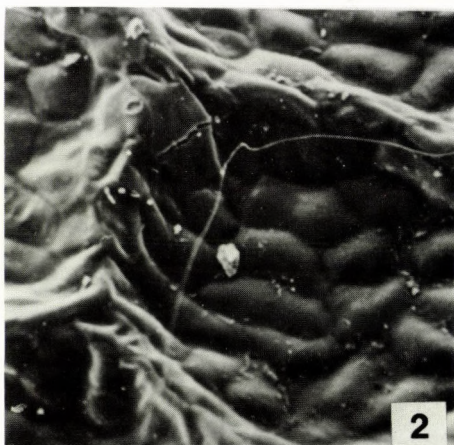
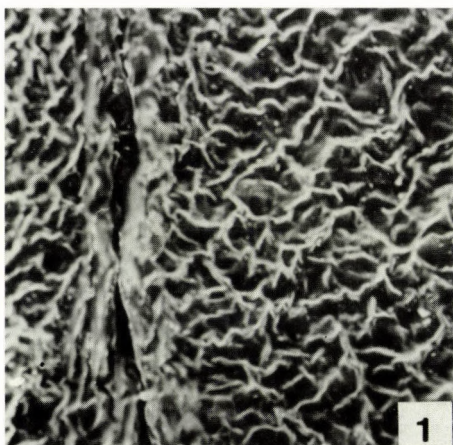
On the abaxial (dorsal) side, cells in the middle part are elongate, narrowly oblong, with transversal strongly thickened walls (300 x). Cells near to the margin are elongate, never oblong and without transversal thickening. On the adaxial (ventral) side cells are broader and larger (400 x) with scattered anomocytic stomata (Plate III, Fig. 5). The membranous margin consist of needle-shaped acerosus appendices (300 x). Apical parts are ciliate and papillate. Papillae are situated below the tip of the abaxial side. Ciliae only at the apex.

Disc flowers

Corolla lobes apically tapering, revolute, tips nailed. Nails built up of little sphaeroid and irregularly shaped granules. At the apical part of the corolla cells are parenchymatic or slightly elongate, smaller than on the abaxial side. Longitudinal and transversal walls strongly thickened. On the abaxial side, cells are very oblong with waved and thickened transversal walls.

Anthers

Cells of the tapetum wall are usually 3-, 4-, and/or 5-angled, with different size and shape.



Filaments

Under the connectivum and above the basal part, cells are anisodiametric parenchymatic and elongate. At the middle part, an articulation is formed by isodiametric parenchymatic cells, with waved and thickened walls (Plate IV, Fig. 11).

Pollen

Tricolporate, lophate type with protruding large colpi, densely tuberculate exine with big triangular spines and punctitegillate perforations at the base (Plate IV, Fig. 7).

Style

Base bulbous; cells are parenchymatic becoming elongate towards the apex of the bulb. Walls of the parenchymatic cells are thickened, the transversal walls waved. On the style branches, the lower side is papillate, tip obtuse surrounded by a tuft of longer cilia. The surface of the stigma is deeply cracked, continuing along the surface of the style.

Achene

It has a few layered wall. The outer cell layer of the ribs contains cells with less thickened walls, than the inner cell layer. Between the ribs the outer layer has parenchymatic cells with linear and waved longitudinal and transversal walls. The internal layer consists of elongate cells with strongly thickened longitudinal walls. The rim contains oval, polygonal and elongate cells (Plate IV, Fig. 13).

Pappus

One thread of pappus is composed of many bristles. One bristle has four different recognizable parts: the base, the style, the subapical part and the spine. Spines short, triangular (Plate IV, Fig. 9).

Plate III.

Fig. 1. Adaxial surface of the leaves in Herodotia haitiensis (SEM 100x)

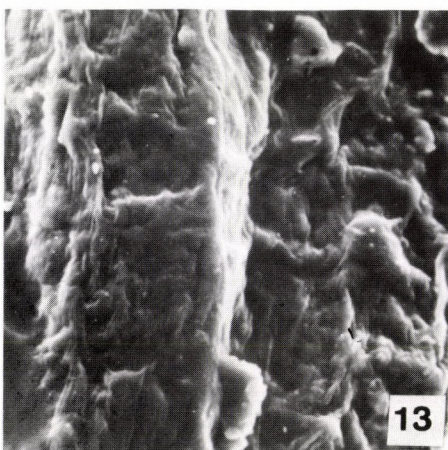
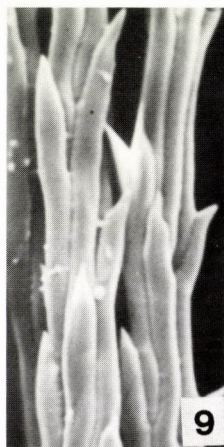
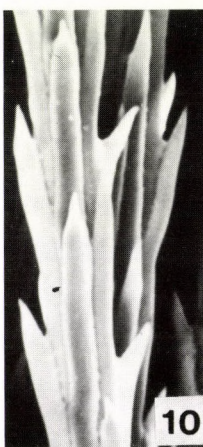
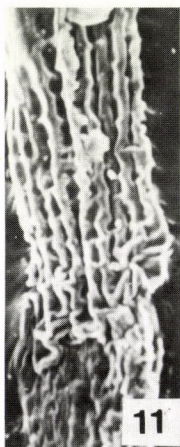
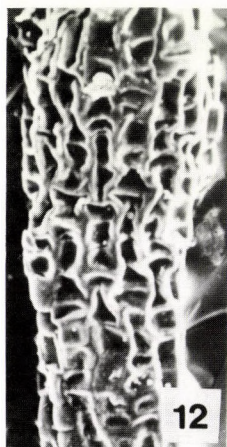
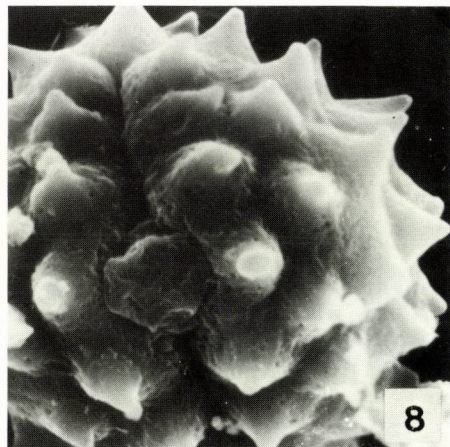
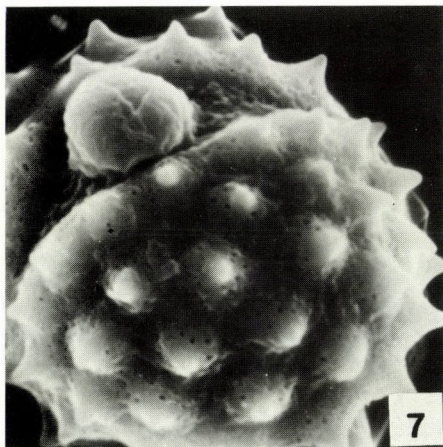
Fig. 2. Adaxial surface of the leaf epidermis in Ekmaniopappus mikanioides (SEM 200x)

Fig. 3. Anomocytic stoma on the abaxial surface on the leaf of Herodotia haitiensis (SEM 2000x)

Fig. 4. Cyclocytic stoma on the abaxial leaf surface of Ekmaniopappus mikanioides (SEM 2000x)

Fig. 5. Involucral bract, adaxial surface with stoma in Herodotia haitiensis (SEM 400x)

Fig. 6. Involucral bract, adaxial surface with bundles and without stomata in Ekmaniopappus mikanioides (SEM 1000x)



Leaves

Adaxial surface consists of small multiangular cells with strong crest-shaped thickenings on the outer wall. Nerves are narrowly impressed in deep lacunes (Plate III, Fig. 1). Abaxial surface with anomocytic stomata (Plate III, Fig. 3), situated on the bottom of the shallower or deeper lacunes; they are mostly sunken into the surface. Veins are sunken, frequently accompanied by stomata.

Ekmaniopappus mikanioides (Urb. & Ekm.) Borhidi

Bracts

Cells on the abaxial side elongate with thickened longitudinal and transversal walls. In the middle part they are linear and cylindric, in the marginal regions more flattened and elongate, non linear. On the adaxial side cells are oblong, flattened with moderately thickened longitudinal walls in the middle part cells are linear, cylindric and emergent, forming costa-like prominent nerves. The adaxial surface without stomata. The tip ends in long sweeping cilia, below the tip with a densely papillate area on the abaxial side (Plate III, Fig. 6).

Subinvolucral bracteoles

Cells of the adaxial side are oblong, parenchymatic, and elongate on the abaxial side, without thickenings on the walls. Tip with long conic cilia, without papillate zone.



Plate IV

Fig. 7. Pollen grain of Herodotia haitiensis (SEM 3000x)

Fig. 8. Pollen grain of Ekmaniopappus mikanioides (SEM 3000x)

Fig. 9. Pappus of Herodotia haitiensis (SEM 200x)

Fig. 10. Pappus of Ekmaniopappus mikanioides (SEM 200x)

Fig. 11. Middle part of the filament of Herodotia haitiensis, with articulation (SEM 200x)

Fig. 12. Middle part of the filament of Ekmaniopappus mikanioides without articulation (SEM 200x)

Fig. 13. Rib and vallecule of the achene in Herodotia haitiensis (SEM 600x)

Fig. 14. Knobs of the vallecular zone in the achene of Ekmaniopappus mikanioides (SEM 1500x)

Ray flowers

Corolla lobe consists of oblong cells on the adaxial side. Tip recurved and nailed. Nail consists of flattened, disciform granules with oblong, cells thickened on both the longitudinal and transversal walls.

Style of disc flowers

The base is bulbous like in Herodotia, but the cells are more elongate and the longitudinal walls thicker. Style branches without papillae on the abaxial surface, only the obtuse tip is papillate. Stigmatic area on the adaxial side shallowly grooved.

Style of ray flowers

Branches shorter than in the disc flowers. Only the tip of the obtuse style bears papillae. Stigma shallowly grooved.

Anthers

Elongated with collar. Apical appendix oblong-ovate with thickened margin; basal appendix short, acute.

Filaments

Shorter and thicker than in Herodotia, articulated at or near to the base. Cells are parenchymatic, more or less isodiametric with thickened walls. Cells of the articulation are similar but with thinner walls than in the upper part of the filament (Plate IV, Fig. 12).

Achene

Cells of the carpopodium oblong-elongate at the base and short-polygonal above. Cells of the ribs are elongate with thickened longitudinal walls. In the vallecule between the ribs cells bear little knobs. Each knob wears two or three erect hairs (Plate IV, Fig. 14).

Pappus

Structure is similar to that of Herodotia, but the spines of the hairs are 1.5-2 times longer, erect and lanceolate (Plate IV, Fig. 10).

Pollen

Tricolporate, colpus not protruding exine densely tuberculate with longer, mammiform apiculate-tipped spines. Punctitegillate perforations less frequent than in Herodotia and concentrated to the middle zone of the spines (Plate IV, Fig. 12).

Leaves

Prominent veins forming areoles on the adaxial surface. Cells varied in form and size with prominent horizontal and sunken perpendicular walls, without cuticular ornamentation (Plate III, Fig. 2). Cyclocytic stomata situated on the abaxial surface. Accessory cells rounded, mostly emerged (Plate III, Fig. 4).

Acknowledgement

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REFERENCES

- Borhidi, A. (1985): Phytogeographic survey of Cuba, I. The phytogeographic characteristics and evolution of the flora of Cuba. Acta Bot. Hung. **31**: 3—34.
- Borhidi, A. (1991): Phytogeography and Vegetation Ecology of Cuba. Akadémiai Kiadó, Budapest, 858 pp.
- Borhidi, A., Gondár, E., Kiss, T. (1989): Ekmaniopappus, separation of a new genus of Senecioneae (Asteraceae) based on floral anatomical evidences. 5th Hung. Plant Anat. Symp. Szeged. Abstracts. p. 12.
- Cuatrecasas, J. (1981): Studies in neotropical Senecioneae II. Transfers to genus Pentacalia of north Andean species. Phytologia **49**: 241—260.
- Jimenez, J. J. (1977): Una nueva compuesta para la Isla de Santo Domingo (Hispaniola). Coll. Conf. Acad. Cien. Rep. Domin. **2**: 7—18.
- Moscoso, R. M. (1943): Catalogus Florae Domingensis, New York.
- Nordenstam, B. (1978): Taxonomic studies in the tribe Senecioneae (Compositae). Opera Bot. **44**: 1—83.
- Robinson, H., Cuatrecasas, J. (1978): A review of the Central American species of Pentacalia (Senecioneae, Asteraceae). Phytologia **40**: 37—50.
- Urban, I. (1926a): Plantae haitienses II. Ark. Bot. **20A** (5): 1—68.
- Urban, I. (1926b): Plantae haitienses III. Ark. Bot. **20A** (15): 1—95.

PHYTOPLANKTON STUDIES IN THE KESZTHELY BAY
(LAKE BALATON, HUNGARY, 1983-1987)

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The eutrophication of Lake Balaton, the largest shallow lake of Central Europe has accelerated for the last fifteen years. In the Keszthely Bay, the most severely affected western basin of the Lake, algological and chlorophyll-a concentration studies — the latter indicating the trophic level — have been carried out since 1982. The samples were taken 50 m off the shore on beaches indicated in the sketch map. The maxima of chlorophyll-a concentration are contained in Table 2. Between 1983 and 1985 the maxima exceeded $100 \text{ mg} \cdot \text{m}^{-3}$ at all sites of sampling; the concentration was outstandingly high ($230 \text{ mg} \cdot \text{m}^{-3}$) in 1986 on the Gyenesdiás beach. On the basis of the concentration averages the highest degree of eutrophication was also found in 1986. Between 1983 and 1985 the water was meso-eutrophic on all beaches. In 1986 the water of the Bay was eutrophic, while in 1987 it proved again to be meso-eutrophic. The total number of algae was high every year, with a maximum ranging from 24.1 to $80.3 \cdot 10^6 \text{ ind} \cdot \text{dm}^{-3}$. The highest value was obtained in 1986; it reached the so far largest 1982 number of algae. By qualitative analyses 406 taxa were identified between 1983 and 1985 (Cyanophyta: 46, Euglenophyta: 16, Xanthophyceae: 1, Chrysophyceae: 6, Bacillariophyceae: 225, Pyrrophyta: 8, Volvocales: 2, Chlorococcales: 88, Ulotrichales: 1, Desmidiaceae: 13) of which 11 taxa are new for Hungary and 11 are new for Lake Balaton alone. Seasonal succession in the phytoplankton is characterized by the dominance of diatoms (Cyclotella spp., Stephanodiscus spp., Nitzschia acicularis) in spring, while from the end of July the filamentous heterocystic blue algae (Aphanizomenon flos-aquae f. klebahnii, Anabaenopsis raciborskii) multiply so much as causing algal bloom every summer. The stability of the taxa is expressed by values of constancy. The taxa of constancy 4 and 5 are stable members of the Keszthely Bay, they only make 1-5 per cent of the total. Most taxa (70-77%) are of constancy 1, that is they occur in less than 20 per cent of the total number of samples. The water of the Bay is characterized by a very high level of eutrophy with a rich phytoplankton.

Keywords: Keszthely Bay, phytoplankton, phytoplankton composition, qualitative analysis, quantitative analysis, seasonal succession, taxon, eutrophy, water bloom

Introduction

The process of eutrophication in Lake Balaton has become faster for the last fifteen years. In the Keszthely Bay, the western basin of the Lake, the high rate of eutrophication caused algal bloom (TAMÁS 1966, 1974, 1975; HORTOBÁGYI 1977; VÖRÖS 1980). Because of the acceleration of the process an

ever increasing number of institutions carry out investigations of the quality of water and the extent of alga formation. The West-Transdanubian Environment Protection- and Water Management Directorate has carried out chemical and biological analyses in the littoral zone and middle of the Keszthely Bay. The results obtained between 1972 and 1976 were published earlier (LENTI and VÍZKELETY 1976; VÍZKELETY and LENTI 1979). In 1979 the alga examinations were completed by measuring the primary production on the areas of dredging at Keszthely and Szigliget (VÖRÖS *et al.* 1983).

In the field of phytoplankton research important work was earlier done by TAMÁS (1967, 1969, 1972, 1975) at the full length of Lake Balaton. Recently VÖRÖS (1985, 1987, 1988), G. TÓTH and PADISÁK (1978, 1986), GORZÓ and KISS (1984, 1985) have published papers on the phytoplankton of the Lake and on its changes in space and time. The results of examinations of the extraordinary algal bloom in 1982 were published in the Botanikai Közlemények (VÍZKELETY 1987--1988). Beside the phytoplankton studies the examination of algal coating and of algae living in the sediment has been pushed into the background. Orientative algological studies on the bed silt of Lake Balaton were earlier carried out by TAMÁS (1966, 1968). In the framework of a periphyton research started in the seventies UHERKOVICH (1979, 1984, 1987) discussed the algae living on the surface of silt and those forming coating on the plants for the entire area of Lake Balaton.

Present paper gives account of the continuation of the algological studies started in 1982 on the beaches of the Keszthely Bay, reporting the 1983--1987 results. In the course of our work we examined the composition, succession and quantity of the phytoplankton. We completed the algological work by measuring the chlorophyll-a concentration, so as to obtain a more accurate picture of the trophity conditions of the Keszthely Bay.

Material and Methods

The samples were taken every two weeks from May to September, on the areas of the beaches indicated on the sketch map No. 1, some 50 metres off the shore from the upper 20 cm layer of the water column.

The samples were fixed with Lugol solution on the spot. Both the alga counting and the chlorophyll-a determination were carried out after FELFÖLDY (1980). The diatoms were identified in preparations imbedded in Styrax resin after destruction in hydrogen peroxide, and microphotographs were taken of the most characteristic ones. The taxa were identified with the aid of the following taxonomic keys: CLEVE-EULER 1951--55; Ettl 1978, 1983; KOMAREK and FOTT 1983; KRAMMER and LANGE-BERTALOT 1986.



KESZTHELY

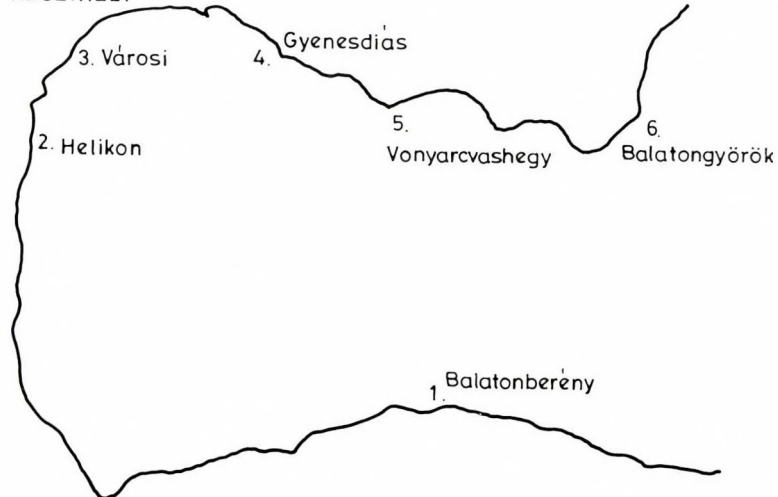


Fig. 1. Sketch map of sampling sites

Table 1

List of species with the values of constancy and highest frequency

Taxa	Constancy			Highest relative frequency					
	1983	1984	1985	1983	1984	1985	Sites of sampling		
				1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.			
CYANOPHYTA									
<u>Anabaena aphanizomenoides</u> FORTI	2	1	2	2 2 3 4 4 4	1 1 1 1 1 1	4 4 4 4 4 4			
<u>Anabaena cylindrica</u> LEMM.	1	-	1	- - - 1 - -	- - - - - -	- - - 1 1 -			
<u>Anabaena scheremetievii</u> ELENKIN	1	-	1	1 - 1 1 1 1	- - - - - -	1 1 1 1 1 1			
<u>Anabaena spiroides</u> KLEB.	2	1	2	4 4 4 4 4 4	1 1 1 1 1 1	4 4 4 4 4 4			
<u>Anabaena variabilis</u> KÜTZ.	1	-	-	1 1 - 1 - -	- - - - - -	- - - - - -			
<u>Anabaenopsis hungarica</u> HALÁSZ	-	-	2	- - - - - -	- - - - - -	1 1 1 1 1 1			
<u>Anabaenopsis raciborskii</u> WOLOSZ.	3	3	4	4 4 4 4 4 4	4 4 4 4 4 4	4 4 4 4 4 4			
<u>Aphanizomenon flos-aquae</u> f. <u>klebahnii</u> ELENK.	3	3	4	4 4 3 4 4 4	4 4 4 4 4 4	4 4 4 4 4 4			
<u>Aphanizomenon issatschenkoi</u> (USS.)PROSCH.-LAVR.	2	1	2	2 2 2 3 3 2	1 1 1 1 1 1	4 4 4 4 4 4			
<u>Aphanocapsa elachista</u> W. et G.S.WEST	1	-	1	- 1 1 1 - -	- - - - - -	- - 1 1 - -			
<u>Aphanothece clathrata</u> W. et G.S.WEST	-	1	-	- - - - - -	1 1 1 - 2 1	- - - - - -			
<u>Aphanothece clathrata</u> W. et G.S.WEST var. <u>media</u> HALÁSZ	3	-	-	4 4 3 2 3 4	- - - - - -	- - - - - -			
* <u>Aphanothece stagnina</u> (SPRENG.)A.BR.	1	-	-	- - 1 - - -	- - - - - -	- - - - - -			
<u>Chroococcus minor</u> (KG.)NAEG.	1	-	-	- - - - 1 -	- - 1 - - -	- - - - - -			
<u>Chroococcus turgidus</u> (KG.)NAEG.	1	-	-	- 1 - 1 - -	- - - - - -	- - - - - -			
<u>Coelosphaerium hungaricum</u> (HORTOB.)FELF.	1	1	-	1 2 3 - - -	1 - - - - 1	- - - - - -			
<u>Coelosphaerium naegelianum</u> UNGER.	1	-	-	1 1 1 1 1 -	- - - - - -	- - - - - -			
<u>Dactylococcopsis acicularis</u> LEMM.	1	1	1	- - 1 1 1 -	- - 1 - - -	- - 1 - - -			
<u>Gomposphaeria aponina</u> KG.	-	1	-	- - - - - -	- 1 - - - -	- - - - - -			
<u>Gomposphaeria lacustris</u> CHODAT	4	4	4	1 1 2 2 2 1	4 4 2 4 4 4	3 3 2 2 3 3			
<u>Gomposphaeria lacustris</u> CHODAT var. <u>compacta</u> LEMM.	-	2	-	- - - - - -	2 3 3 4 4 4	- - - - - -			
<u>Lyngbya circumcreta</u> G.S.WEST	1	4	4	1 1 2 1 1 1	2 1 1 1 2 2	1 1 2 2 1 1			
<u>Lyngbya lagerheimii</u> (MÖB.)GOM.	1	-	-	- - - 1 - 1	- - - - - -	- - - - - -			
<u>Lyngbya limnetica</u> LEMM.	4	5	4	3 3 3 3 2 3	3 3 4 4 4 4	4 4 4 4 4 4			
<u>Merismopedia glauca</u> (EHR.)NAEG.	1	1	1	1 - 1 - - -	- - - 1 - -	1 - - - 1 -			
<u>Merismopedia marssonii</u> LEMM.	-	1	-	- - - - - -	- - - 1 - -	- - - - - -			
<u>Merismopedia minima</u> BECK.	2	3	2	1 2 2 2 1 2	2 3 2 1 2 1	1 1 1 1 1 1			
<u>Microcystis aeruginosa</u> KG.	1	1	-	2 1 2 2 2 1	- - 1 1 1 -	- - - - - -			
<u>Microcystis flos-aquae</u> (WITTR.) KIRCHN.	1	1	1	1 1 - - - 1	1 - - - - 1	- - 1 - - -			
<u>Microcystis holsatica</u> LEMM.	-	2	2	- - - - - -	1 1 1 1 1 1	1 1 1 1 1 1			
<u>Microcystis holsatica</u> var. <u>minor</u> LEMM.	1	2	-	- 1 - - - -	1 1 1 1 1 1	- - - - - -			
<u>Microcystis minutissima</u> W.WEST	4	3	5	4 4 4 4 4 4	4 4 4 4 4 4	2 2 4 2 2 2			
<u>Microcystis stagnalis</u> LEMM.	-	1	-	- - - - - -	1 - - - - -	- - - - - -			
<u>Oscillatoria amphibia</u> AGH.	-	1	-	- - - - - -	1 - 1 - 1 1	- - - - - -			
<u>Oscillatoria formosa</u> BORY	1	-	-	- - 1 - - -	- - - - - -	- - - - - -			
<u>Oscillatoria granulata</u> GARDNER	-	-	1	- - - - - -	- - - - - -	- - 1 - - -			

Table 1 (cont.)

Taxa	Constancy			Highest relative frequency					
	1983	1984	1985	1983	1984	1985	Sites of sampling		
				1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.			
<u>Oscillatoria limnetica</u> LEMM.	-	1	-	- - - - -	- - 1 - -	- - - - -			
<u>Oscillatoria putrida</u> SCHMIDLE	1	-	-	- - - - 2	- - - - -	- - - - -			
<u>Oscillatoria tenuis</u> AGH.	1	1	-	- 1 1 - -	- - 1 1 1 -	- - - - -			
<u>Pseudanabaena balatonica</u> SCHERFF. et KOL	-	1	-	- - - - -	- - - - 1	- - - - -			
<u>Pseudanabaena catenata</u> LAUTERB.	-	1	-	- - - - -	1 - - - -	- - - - -			
<u>Raphidiopsis mediterranea</u> SKUJA	1	1	1	- 2 2 3 3 2	1 1 1 - 1 -	1 1 1 - 1 -			
<u>Rhabdoderma lineare</u> SCHMIDLE et LAUTERB.	1	-	-	- 1 - 1 - -	- - - - -	- - - - -			
<u>Rhabdoderma minima</u> LAUTERB.	1	-	-	- - - 1 -	- - - - -	- - - - -			
<u>Romeria elegans</u> (KOCZW.)WOL.	1	1	1	1 2 2 1 1 -	2 1 - 1 1 -	1 1 - - -			
<u>Romeria gracilis</u> KOCZW.	-	1	1	- - - - -	- - 1 - 1 1	1 - 1 1 1 -			
EUGLENOPHYTA									
<u>Colatium vesiculosum</u> EHR.	1	-	-	1 - - - -	- - - - -	- - - - -			
<u>Euglena acus</u>	1	1	1	- - 1 1 1 1	- - 1 1 1 1	1 - 1 1 - -			
<u>Euglena ehrenbergii</u> KLEBS	1	-	-	- 1 - - -	- - - - -	- - - - -			
<u>Euglena oblonga</u> SCHMITZ	-	1	-	- - 1 - -	- - - - -	1 1 - 1 1 -			
<u>Euglena oxyuris</u>	1	-	1	- - 1 - -	- - - - -	1 1 - 1 1 -			
<u>Euglena polymorpha</u> DANG.	1	1	1	- - 1 1 - -	- 1 1 - -	- - 1 - -			
<u>Euglena texta</u> (DUJ.)HÜBN.	-	1	1	- - - - -	1 - - - -	- - 1 1 1 1			
<u>Euglena tripteris</u> (DUJ.)KLEBS	1	1	1	- 1 1 - -	1 - - - -	- - - 1 1 -			
<u>Euglena variabilis</u> KLEBS	-	-	1	- - - - -	- - - - -	1 1 - - 1 -			
<u>Lepocinclis fusiformis</u> (CARTER) LEMM.	-	-	1	- - - - -	- - - - -	- - - - 1 -			
<u>Phacus acuminatus</u> STOKES	1	1	1	- 1 1 - - -	- 1 1 - 1 -	- 1 1 1 1 1			
<u>Phacus curvicauda</u> SWIR.	1	-	-	- 1 1 1 - -	- - - - -	- - - - -			
<u>Phacus longicauda</u> var. <u>tortus</u> LEMM.	1	1	1	1 1 - 1 1 -	1 - - - -	- - 1 1 1 1			
<u>Phacus orbicularis</u> HÜBN.	-	1	-	- - - - -	1 - - - -	- - - - -			
<u>Phacus pleuronectes</u> (O.F.MÜLL.)DUJ.	1	-	-	- 1 1 - 1 -	- - - - -	- - - - -			
<u>Phacus pyrum</u> (EHR.)STEIN	1	2	2	1 1 2 1 1 -	1 1 1 1 1 -	1 1 1 1 1 1			
CHRYSOPHYTA									
Xanthophyceae									
<u>Ophiocytium capitatum</u> WOLLE	1	-	-	- 1 - 1 - -	- - - - -	- - - - -			
Crysophyceae									
<u>Anthophysa vegetans</u> (O.F.M.)STEIN	-	1	-	- - - - -	- - - 1 - -	- - - - -			
<u>Chrysococcus biporus</u> SKUJA	1	1	1	1 - - - -	1 - - - -	1 - - 1 - -			
<u>Chrysococcus triporus</u> MATVIENKO	-	-	1	- - - - -	- - - - -	1 - - - -			
<u>Kephyrion rubri-claustri</u> CONRAD	-	1	1	- - - - -	- 1 - - -	- - 1 - -			
<u>Pseudokephyrion entzii</u> CONRAD	1	1	-	1 - - - -	- 1 - 1 1 -	- - - - -			
<u>Synura uvella</u> EHR.	-	-	1	- - - - -	- - - - -	- - - 1 - -			

Table 1 (cont.)

Taxa	Constancy			Highest relative frequency		
	1983	1984	1985	1983	1984	1985
	Sites of sampling 1.2.3.4.5.6. 1.2.3.4.5.6. 1.2.3.4.5.6.					
Bacillariaphyceae (Diatomeae)						
<i>Achnanthes affinis</i> GRUN.	1	-	-	- - - 1 1 1	- - - - -	- - - - -
<i>Achnanthes clevei</i> GRUN.	1	1	1	- - - 1 1 1	1 1 1 - 1 1	1 1 1 - -
<i>Achnanthes clevei</i> var. <i>rostrata</i> HUST.	-	1	1	- - - - -	1 1 - - -	1 1 - 1 4 1
<i>Achnanthes dispar</i> CL.	-	-	1	- - - - -	- - - - -	- - - - -
<i>Achnanthes hustedtii</i> BILY et MARVAN	-	-	1	- - - - -	- - - - -	- - - - 1 -
<i>Achnanthes lanceolata</i> (BRÉB.)GRUN.	1	1	1	- - 1 1 - 1	1 - 1 - - -	- - 1 1 1 -
<i>Achnanthes lanceolata</i> (BRÉB.)GRUN. var. <i>rostrata</i> (ØSTRUP)HUST.	-	1	-	- - - - -	- - 1 - - -	- - - - -
<i>Achnanthes lanceolata</i> f. <i>ventri-</i> <i>cosa</i> HUST.	-	-	1	- - - - -	- - - - -	- - - 1 - -
<i>Achnanthes linearis</i> (W.SM.)GRUN.	-	1	1	- - - - -	- - - 1 - -	1 - - 1 1 1
+ <i>Amphora commutata</i> GRUN.	-	-	1	- - - - -	- - - - -	- - - 1 - -
<i>Amphora libyca</i> EHR.	3	3	4	2 1 2 1 1 1	3 2 2 2 1 1	3 1 2 2 1 1
<i>Amphora ovalis</i> KÜTZ.	2	3	4	2 1 1 1 1 1	1 1 1 1 1 1	2 1 1 1 1 1
* <i>Amphora thumensis</i> (A. MAYER) CL.EUL.	-	1	-	- - - - -	1 - 1 - - -	- - - - -
<i>Anomoeoneis sphaerophora</i> (KÜTZ.)PFITZ.	-	-	1	- - - - -	- - - - -	1 - - 1 1 -
<i>Asterionella formosa</i> HASS.	4	1	1	4 4 4 4 4 4	- - - 1 - -	1 - - 1 - -
<i>Attheya zachariasii</i> BRUN.	2	2	1	3 3 1 2 2 2	- - 1 1 1 1	- - 1 1 1 -
<i>Bacillaria paradoxa</i> GMELIN	-	-	1	- - - - -	- - - - -	1 - - - - -
+ <i>Bacillaria paradoxa</i> var. <i>tumidula</i> GRUN.	-	-	1	- - - - -	- - - - -	- - 1 - - -
<i>Caloneis amphisbaena</i> (BORY)CL.	1	1	1	1 1 - 1 1 -	1 1 1 1 - 1	1 1 - - 1 -
<i>Caloneis bacillum</i> (GRUN.)MER.	1	-	1	- - - - - 1	- - - - -	1 1 - - - -
<i>Caloneis schumanniana</i> var. <i>biconstricta</i> GRUN.	-	-	1	- - - - -	- - - - -	- - 1 - - -
<i>Caloneis silicula</i> (EHR.)CL.	1	1	1	1 1 - - - -	- 1 1 1 1 1	1 1 1 1 1 -
<i>Caloneis silicula</i> var. <i>truncatula</i> GRUN.	1	-	-	- - - - 1 -	- - - - -	- - - - -
<i>Cocconeis diminuta</i> PANT.	1	1	1	- - 1 1 1 -	- 1 1 1 1 1	1 1 1 1 1 -
<i>Cocconeis disculus</i> (SCHUM.)CL.	1	-	1	- - - - 1 1	- - - - -	1 1 1 - 1 1
<i>Cocconeis pediculus</i> EHR.	2	2	2	- 1 1 1 1 1	1 1 1 1 1 1	1 1 2 1 1 1
<i>Cocconeis placentula</i> EHR.	2	1	2	1 1 1 1 1 1	1 1 1 1 1 -	1 1 2 1 1 1
<i>Cocconeis placentula</i> var. <i>euglypta</i> (EHR.)CL.	1	1	1	- - - 1 1 1	- 1 1 1 1 1	- 1 1 1 1 1
<i>Cyclostephanos dubius</i> (FRICKE) ROUND	4	2	3	4 4 4 4 4 4	1 1 2 2 2 1	2 2 2 1 1 1
<i>Cyclotella atomus</i> HUST.	1	2	1	1 1 1 - 1 -	1 1 1 1 1 1	- 1 - - - -
<i>Cyclotella comta</i> (EHR.)KÜTZ.	1	3	5	1 - 1 1 1 1	1 2 1 2 2 1	4 4 4 4 4 4
<i>Cyclotella meneghiniana</i> KÜTZ.	2	3	2	2 2 2 1 1 1	1 1 1 1 2 1	1 1 1 1 - 1
<i>Cyclotella ocellata</i> PANT.	2	2	5	1 - 1 1 1 1	1 3 2 3 1 1	4 4 4 4 4 4
<i>Cyclotella pseudostelligera</i> HUST.	1	2	3	1 1 1 - - -	1 1 1 2 2 1	4 4 2 4 4 2
<i>Cyclotella stelligera</i> CL. et GRUN.	1	-	-	1 1 1 - - -	- - - - -	- - - - -

Table 1 (cont.)

Taxa	Constancy			Highest relative frequency					
	1983	1984	1985	1983	1984	1985	Sites of sampling		
				1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.
<u>Cymatopleura angulata</u> GREV.	-	-	1	- - - - -	- - - - -	- 1 1 - - 1			
<u>Cymatopleura elliptica</u> (BREB.)W.SM.	1	1	1	- 1 1 1 1 -	1 - 1 - - -	1 - 1 - 1 1			
<u>Cymatopleura elliptica</u> var. <u>hibernica</u> (W.SM.)HUST.	-	1	1	- - - - -	- - 1 - - -	- - - - -			
<u>Cymatopleura elliptica</u> var. <u>nobilis</u> (HANTZSCHE)HUST.	-	1	1	- - - - -	- - - - -	- - - 1 - -			
<u>Cymatopleura solea</u> (BREB.)W.SM.	2	1	2	1 2 1 1 1 1	1 1 - 1 1 1	1 1 1 1 1 1			
<u>Cymatopleura solea</u> var. <u>apiculata</u> (W.SM.)RALFS	1	-	-	- 2 - - - 1	- - - - -	- - - - -			
<u>Cymatopleura solea</u> var. <u>pfuhlui</u> TORKA	-	1	1	- - - - -	- - - - 1 -	- - - - 1 1			
<u>Cymatopleura solea</u> var. <u>regula</u> (EHR.)GRUN.	-	-	1	- - - - -	- - - - -	- - 1 - - -			
<u>Cymbella affinis</u> KÜTZ.	-	1	1	- - - - 1 -	1 1 1 - - 1	1 1 1 1 1 -			
<u>Cymbella amphicephala</u> NAEG.	1	1	-	- - - 1 - -	- - 1 - - -	- - - - -			
<u>Cymbella aspera</u> (EHR.)CL.	1	1	1	- - - - -	- - - - 1 -	- 1 - - - -			
<u>Cymbella cistula</u> (HEMP.)GRUN.	1	1	1	- 1 - 1 - -	1 - - - -	- - - 1 - -			
<u>Cymbella cymbiformis</u> (AG.)KÜTZ.V.H.	1	1	1	- - 1 - 1 -	- - - 1 - -	1 1 - - 1 -			
<u>Cymbella ehrenbergii</u> KÜTZ.	1	-	1	- - - - 1 1	- - - - -	- 1 - 1 - 1			
<u>Cymbella elginensis</u> KRAMMER	-	1	-	- - - - -	- - - 1 - -	- - - - -			
<u>Cymbella elliptica</u> var. <u>nobilis</u> (HANTZSCH)HUST.	-	1	-	- - - - -	- - - 1 - -	- - - - -			
<u>Cymbella lanceolata</u> (EHR.)V.H.	-	1	1	- - - - -	1 - - 1 - 1	1 1 - 1 1 -			
<u>Cymbella naviculiformis</u> AUERSW.	-	1	1	- - - - -	1 - - - -	- 1 - - - -			
<u>Cymbella parva</u> (W.SM.)CL.	-	1	-	- - - - -	- - 1 - - 1	- - - - -			
<u>Cymbella prostrata</u> (BERKELEY)CL.	-	1	2	- - - - -	- - - 1 - 1	1 1 1 1 1 1			
<u>Cymbella pusilla</u> GRUN.	-	1	1	- - - - -	- - - - 1 -	- - - - 1 -			
<u>Cymbella silesiaca</u> BLEISCH IN RABENHORST	1	-	2	1 1 1 1 - 2	- - - - -	1 1 1 1 1 1			
<u>Diatoma elongatum</u> (LYNGB.)AG.	1	1	1	- - - - - 1	1 - - - 1 1	- - - 1 1 1			
<u>Diatoma elongatum</u> var. <u>tenue</u> (A.G.)V.H.	-	-	1	- - - - -	- - - - -	- - - - 1 -			
<u>Diatoma hiemale</u> (LYNGB.)HEIB.	1	-	2	1 1 - - -	- - - - -	1 1 1 - 3 1			
<u>Diatoma vulgare</u> BORY	1	1	2	- - - - 2 3	- - - - - 1	- 1 1 - 1 1			
<u>Diatoma vulgare</u> var. <u>lineare</u> GRUN.	1	1	1	1 1 1 1 1 1	1 1 1 - - -	1 1 1 1 1 -			
<u>Diatoma vulgare</u> var. <u>productum</u> GRUN.	1	1	1	1 - - 1 - 1	1 - - - -	- - - - 1 1			
<u>Diploneis oculata</u> (BRÉB.)CL.	-	-	1	- - - - -	- - - - -	1 - - - 1 -			
<u>Diploneis ovalis</u> (HILSE)CL.	-	-	1	- - - - -	- - - - -	1 - - - -			
<u>Diploneis smithii</u> var. <u>laevis</u> J.-DANNF.	1	1	1	1 - 1 1 1 1	1 1 1 - - -	1 1 1 1 1 -			
<u>Epithemia intermedia</u> FRICKE	1	-	-	- - - - 1 -	- - - - -	- - - - -			
* <u>Epithemia mülleri</u> FRICKE	-	1	-	- - - - -	1 - - - -	- - - - -			
<u>Epithemia sorex</u> KÜTZ.	1	1	1	1 1 1 1 1 -	1 1 - 1 1 -	1 1 - 1 - 1			
<u>Epithemia turgida</u> (EHR.)KÜTZ.	1	1	1	- - - - - 1	- - 1 - - -	- - - 1 1 1			
<u>Epithemia turgida</u> var. <u>granulata</u> (EHR.)GRUN.	1	1	1	- - - - 1 -	- 1 - 1 - -	- - - 1 - -			

Table 1 (cont.)

Taxa	Constancy			Highest relative frequency											
	1983	1984	1985	1983	1984	1985	Sites of sampling								
				1	2	3	4	5	6	1	2	3	4	5	6
<u>Epithemia zebra</u> (EHR.)KÜTZ.	-	-	1	-	-	-	-	-	-	-	1	1	-	1	-
<u>Epithemia zebra</u> var. <u>porcellus</u> (KÜTZ.)GRUN.	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
<u>Epithemia zebra</u> var. <u>saxonica</u> (KÜTZ.)GRUN.	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-
<u>Eunotia valida</u> HUST.	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-
<u>Fragilaria bicapitata</u> MAYER	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-
<u>Fragilaria brevistriata</u> GRUN.	2	2	2	1	-	1	1	1	1	1	1	1	1	1	1
<u>Fragilaria capucina</u> DESM.	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
<u>Fragilaria construens</u> (EHR.)GRUN.	3	3	4	4	4	3	1	1	2	4	4	1	1	1	1
* <u>Fragilaria construens</u> var. <u>triundulata</u> REICH.	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-
<u>Fragilaria crotonensis</u> KITT.	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-
<u>Fragilaria inflata</u> (HEID.)HUST.	3	3	4	4	3	3	2	2	2	4	3	1	1	1	1
<u>Fragilaria inflata</u> var. <u>istvanffy</u> (PANT.)HUST.	1	1	1	1	-	-	1	1	1	1	-	-	-	1	1
<u>Fragilaria intermedia</u> GRUN.	1	1	1	-	-	-	1	-	-	-	1	1	1	1	1
<u>Fragilaria pinnata</u> EHR.	3	2	2	4	3	1	2	2	1	2	1	1	1	1	1
<u>Fragilaria vaucheriae</u> (KÜTZ.)BOYE PET.	1	2	1	1	-	1	-	1	1	1	1	2	1	1	1
<u>Frustulia rhomboides</u> (EHR.)DE TONI	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-
<u>Gomphonema acuminatum</u> EHR.	1	-	1	-	-	1	-	1	1	-	-	-	-	-	1
<u>Gomphonema angustatum</u> (KÜTZ.)RABH.	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-
<u>Gomphonema angustum</u> AG.	1	1	1	-	-	-	1	1	-	1	-	-	1	1	1
<u>Gomphonema augur</u> EHR.	-	-	1	-	-	-	-	-	-	-	-	-	1	1	-
<u>Gomphonema longiceps</u> var. <u>subclavatum</u> GRUN.	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-
<u>Gomphonema olivaceum</u> (LYNGB.)KÜTZ.	1	1	1	-	-	-	1	-	-	1	1	1	1	1	1
<u>Gomphonema parvulum</u> (KÜTZ.)GRUN.	1	-	1	-	-	-	1	1	-	-	-	-	-	1	-
<u>Gomphonema parvulum</u> var. <u>micropus</u> (KÜTZ.)CL.	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
<u>Gomphonema tergestinum</u> (GRUN.)FRICKE	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-
<u>Gomphonema truncatum</u> EHR.	-	1	1	-	-	-	-	-	-	-	-	1	1	-	-
<u>Gomphonema ventricosum</u> GREG.	-	1	1	-	-	-	-	-	-	1	-	-	-	1	-
<u>Gyrosigma acuminatum</u> (KÜTZ.)RABH.	1	1	1	-	-	-	1	1	1	-	-	1	-	-	1
<u>Gyrosigma distortum</u> (W.SM.)CL.	1	1	1	-	1	-	1	-	-	-	1	-	-	1	-
<u>Gyrosigma parkerii</u> (HARR.)ELMORE	1	1	1	-	-	1	1	-	-	-	1	-	1	1	-
<u>Gyrosigma prolongatum</u> (W.SM.)CL.	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-
+ <u>Gyrosigma prolongatum</u> var. <u>closteroides</u> GRUN.	1	-	1	-	1	-	-	-	-	-	-	-	-	1	-
<u>Gyrosigma scalproides</u> (RABH.)CL.	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-
<u>Gyrosigma strigilis</u> (W.SM.)CL.	-	-	1	-	-	-	-	-	-	-	-	-	-	1	1
<u>Melosira distans</u> (EHR.)KÜTZ.	1	1	2	-	-	1	-	-	-	1	2	1	1	1	1
<u>Melosira distans</u> var. <u>lirata</u> (O.MÜLL.)BETHGE	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
<u>Melosira granulata</u> (EHR.)RALFS	3	3	2	4	4	2	4	3	3	2	2	2	2	1	1

Table 1 (cont.)

Taxa	Constancy			Highest relative frequency					
	1983	1984	1985	1983	1984	1985	Sites of sampling		
				1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.			
<u>Melosira granulata</u> var. <u>angus-</u> <u>tissima</u> (O.F.M.)HUST.	4	5	4	4 4 4 4 4 4	1 4 2 2 1 1	1 1 1 2 1 1			
<u>Melosira granulata</u> var. <u>angus-</u> <u>tissima</u> f. <u>curvata</u> O.MÜLL.	3	1	1	4 4 3 4 4 3	- - 1 1 1 1	- 1 - - - 1			
<u>Melosira granulata</u> var. <u>muzza-</u> <u>nensis</u> (MEISF.)HUST.	2	1	1	2 2 3 2 2 1	- - 1 - - -	1 1 - - - -			
<u>Melosira izlandica</u> O.MÜLL.	-	-	1	- - - - - -	- - - - - -	- - 1 - - -			
<u>Melosira izlandica</u> ssp. <u>helve-</u> <u>tica</u> O.MÜLL.	-	1	1	- - - - - -	- 1 - 1 - -	- - 1 - - -			
<u>Melosira varians</u> AG.	-	1	1	- - - - - -	- 1 - - - -	1 1 - 1 1 -			
<u>Meridion circulare</u> AG.	1	-	-	- - - 1 - -	- - - - - -	- - - - - -			
<u>Navicula amphibola</u> CL.	-	1	-	- - - - - -	1 - - - - -	- - - - - -			
<u>Navicula cari</u> EHR.	1	-	-	- 1 - - - -	- - - - - -	- - - - - -			
<u>Navicula capitata</u> EHR. var. <u>capitata</u>	1	2	3	1 1 1 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1			
<u>Navicula capitata</u> var. <u>hungarica</u> (GRUN.)ROSS	1	-	2	- - - 1 - -	- - - - - -	1 1 1 1 1 -			
<u>Navicula capitatoradiata</u> GERMAIN	2	3	3	2 1 2 1 1 1	1 2 1 1 1 1	1 1 2 2 1 2			
<u>Navicula costulata</u> GRUN.	2	1	1	1 1 1 1 1 -	1 - 1 1 1 -	- 1 - 1 1 1			
<u>Navicula cuspidata</u> KÜTZ.	-	1	1	- - - - - -	- - 1 - - -	- - - - - -			
<u>Navicula cuspidata</u> var. <u>ambigua</u> (EHR.)CL.	-	1	-	- - - - - -	- - - 1 - -	- - - - - -			
<u>Navicula elginensis</u> (GREG.)RALFS in PRITCHARD var. <u>elginensis</u>	1	1	1	1 - 1 1 - -	- - 1 - - -	- 1 1 1 - -			
+ <u>Navicula exigua</u> (GREG.)O.MÜLL.	-	1	-	- - - - - -	- - - 1 - -	- - - - - -			
<u>Navicula gastrum</u> (EHR.)KÜTZ.	1	-	1	1 - - - - -	- - - - - -	- - - 1 - -			
<u>Navicula gastrum</u> var. <u>signata</u> HUST.	1	1	1	1 - 1 1 - -	1 1 1 - 1 -	2 1 - - - 1			
<u>Navicula hasta</u> PANT.	-	1	-	- - - - - -	- - - - - 1	- - - - - -			
+ <u>Navicula kotschy</u> GRUN.	-	1	1	- - - - - -	- - - - - -	- 1 - - - -			
<u>Navicula laterostrata</u> HUST.	-	-	1	- - - - - -	- - - - - -	- - - - - 1			
<u>Navicula mutica</u> KÜTZ.	-	1	-	- - - - - -	1 - - - - -	- - - - - -			
<u>Navicula nivalis</u> EHR.	-	1	-	- - - - - -	- - - 1 - -	- - - - - -			
<u>Navicula oblonga</u> KÜTZ.	-	1	1	- - - - - -	1 - - - - -	- 1 - - - -			
<u>Navicula peregrina</u> (EHR.)KÜTZ.	-	1	1	- - - - - -	1 - - - - -	1 - - - - -			
<u>Navicula placentula</u> (EHR.)GRUN.	-	-	1	- - - - - -	- - - - - -	- - - 1 - -			
<u>Navicula placentula</u> f. <u>rostrata</u> MAYER	1	-	1	1 - - 1 - -	- - - - - -	1 - - - - -			
* <u>Navicula platystoma</u> EHR.	-	1	-	- - - - - -	- - - 1 - -	- - - - - -			
<u>Navicula pupula</u> KÜTZ.	2	2	2	1 1 1 1 1 -	1 - 1 1 1 1	1 1 1 1 1 -			
<u>Navicula pupula</u> var. <u>capitata</u> HUST.	-	1	1	- - - - - -	- 1 - - - -	1 - - - - -			
<u>Navicula pupula</u> var. <u>elliptica</u> HUST.	-	-	1	- - - - - -	- - - - - -	- 1 - - - -			
<u>Navicula pupula</u> var. <u>rostrata</u> HUST.	1	-	-	1 - - - - -	- - - - - -	- - - - - -			
<u>Navicula pygmaea</u> KÜTZ.	1	-	1	- - - - 1 -	- - - - - -	- - 1 1 - -			
<u>Navicula radiosa</u> KÜTZ.	1	-	1	1 1 1 - - -	- - - - - -	- 1 - 1 1 -			
<u>Navicula reinhardtii</u> GRUN.	1	1	1	- - - - 1 -	1 - - - - -	1 1 - 1 - -			
<u>Navicula rhynchocephala</u> KÜTZ.	1	1	4	2 2 1 1 1 2	1 2 1 1 1 -	1 2 1 1 1 1			
<u>Navicula scutelloides</u> W.SM.	1	1	2	1 - 1 1 1 -	1 1 - - 1 -	2 1 1 1 1 1			
<u>Navicula tripunctata</u> (O.F.MÜLL.)BORY	2	2	2	- 1 2 - 1 1	- 1 1 1 1 1	1 1 1 1 1 1			

Table 1 (cont.)

Taxa	Constancy			Highest relative frequency					
	1983	1984	1985	1983	1984	1985	Sites of sampling		
				1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.			
<i>Navicula tuscula</i> (EHR.)GRUN.	1	1	1	1 - - 1 1 -	1 1 1 - 1 1	1 1 - 1 - 1			
<i>Navicula viridula</i> KÜTZ.	1	1	1	1 - 1 1 2 -	1 1 1 1 - 1	1 1 1 1 1 1			
<i>Neidium dubium</i> (EHR.)CL.	1	1	1	1 1 - - 1 1	1 1 - - - -	1 1 - 1 - 1			
<i>Neidium iridis</i> (EHR.)CL.	-	-	1	- - - - - -	- - - - - -	1 - - - - 1			
<i>Nitzschia acicularis</i> W.SM.	4	5	5	4 4 4 4 4 4	4 4 4 4 4 4	4 4 4 4 4 4			
<i>Nitzschia actinastroides</i> (LEMM.) VAN GOOR	1	4	2	2 2 3 3 3 -	4 4 4 4 4 4	1 2 1 1 1 1			
<i>Nitzschia amphibia</i> GRUN.	2	2	1	2 3 1 1 1 1	1 1 1 1 1 -	1 1 1 1 - 1			
<i>Nitzschia angustata</i> (W.SM.)GRUN.	-	-	1	- - - - - -	- - - - - -	- 1 - 1 - -			
<i>Nitzschia angustata</i> var. <i>acuta</i> GRUN.	1	-	1	1 - - - - -	- - - - - -	1 - - - - -			
<i>Nitzschia closterium</i> (EHR.)W.SM.	-	-	1	- - - - - -	- - - - - -	1 - - - - -			
<i>Nitzschia commutata</i> GRUN.	1	-	1	- - 1 1 - -	- - - - - -	- 1 - - - -			
<i>Nitzschia dissipata</i> (KÜTZ.)GRUN.	1	-	1	- - - - 1 -	- - - - - -	- - 1 - 1 -			
* <i>Nitzschia dubia</i> W.SM.	1	-	-	- - - 1 - 1	- - - - - -	- - - - - -			
<i>Nitzschia flexa</i> SCHUM.	-	1	-	- - - - - -	- - 1 1 - -	- - - - - -			
<i>Nitzschia hungarica</i> GRUN.	2	1	1	1 1 1 1 1 1	- 1 - - 1 -	- 1 1 1 1 1			
<i>Nitzschia ignorata</i> KRASSKE	-	1	-	- - - - - -	- - - 1 - -	- - - - - -			
<i>Nitzschia kützingiana</i> HILSE	1	2	2	- 1 1 1 1 -	1 1 1 2 1 2	- 1 1 1 1 1			
<i>Nitzschia linearis</i> W.SM.	1	2	3	2 2 1 1 - 1	1 1 1 1 1 -	1 2 2 1 1 1			
<i>Nitzschia lorenziana</i> var. <i>subtilis</i> GRUN.	-	1	-	- - - - - -	- - 1 1 - -	- - - - - -			
<i>Nitzschia palea</i> (KÜTZ.)W.SM.	4	3	3	2 2 3 2 2 2	1 2 2 2 1 1	1 3 2 2 2 2			
<i>Nitzschia palea</i> var. <i>tenuirostris</i> GRUN.	1	1	1	- 1 1 - 1 -	- 1 1 1 - 1	- - 1 - - -			
<i>Nitzschia paleacea</i> GRUN.	-	-	1	- - - - - -	- - - - - -	1 1 - - - -			
* <i>Nitzschia paleaeformis</i> HUST.	-	-	1	- - - - - -	- - - - - -	- - 1 1 - -			
<i>Nitzschia recta</i> HANTZSCH	1	2	3	- - 1 1 1 1	1 2 1 2 1 2	1 1 1 1 1 1			
<i>Nitzschia sigma</i> (KÜTZ.)W.SM.	-	1	1	- - - - - -	- - - - 1 -	- 1 - 1 - 1			
<i>Nitzschia sigmoidea</i> (EHR.)W.SM.	2	2	2	1 2 2 1 1 2	2 1 1 1 1 1	1 1 1 1 1 1			
<i>Nitzschia stagnorum</i> RABH.	1	2	2	1 2 - 2 2 2	1 1 1 2 2 1	1 1 1 1 1 1			
<i>Nitzschia sublinearis</i> HUST.	1	-	1	- 1 1 1 - -	- - - - - -	- 1 1 1 - 1			
<i>Nitzschia thermalis</i> KÜTZ.	-	-	1	- - - - - -	- - - - - -	- 1 - 1 - -			
<i>Nitzschia tryblionella</i> HANTZSCH	1	1	2	- - 1 - - -	1 - - 1 1 1	1 1 1 1 1 1			
<i>Nitzschia tryblionella</i> var. <i>calicla</i> GRUN.	1	1	-	1 - - - - -	- - - 1 - -	- - - - - -			
<i>Nitzschia tryblionella</i> var. <i>debilis</i> (ARN.)MAYER	-	1	1	- - - - - -	- - 1 - - -	- - 1 1 - -			
<i>Nitzschia tryblionella</i> var. <i>levi-</i> <i>densis</i> (W.SM.)GRUN.	-	1	-	- - - - - -	- - - - 1 -	- - - - - -			
<i>Nitzschia tryblionella</i> var. <i>vic-</i> <i>toriae</i> GRUN.	3	1	3	1 1 1 2 1 1	1 1 1 1 1 1	1 1 1 1 1 1			
* <i>Oestrupia bicontracta</i> (ØSTRUP) LANGE-BERTALOT et KRAMMER	-	1	-	- - - - - -	- - - - 1 -	- - - - - -			
<i>Opephora martyi</i> HÉRIB.	2	2	3	1 - 1 1 1 1	1 1 1 1 1 -	1 1 1 1 1 1			
* <i>Pinnularia braunii</i> var. <i>amphi-</i> <i>cephala</i> (MAER.)HUST.	-	-	1	- - - - - -	- - - - - -	- - - - - 1			
<i>Pinnularia globiceps</i> GREG.	-	-	1	- - - - - -	- - - - - -	- - - 1 - -			

Table 1 (cont.)

Taxa	Constancy			Highest relative frequency					
	1983	1984	1985	1983	1984	1985	Sites of sampling		
				1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.			
<i>*Pinnularia lata</i> (BRÉB.)W.SM.	-	1	-	- - - - -	- - - 1 - -	- - - - -			
<i>Pinnularia maior</i> (KÜTZ.)CL.	1	-	-	1 1 1 2 1 1	- - - - -	- - - - -			
<i>Pinnularia microstauron</i> (EHR.)CL.	1	-	1	- - - - 1 -	- - - - -	- - - - - 1			
<i>+Pinnularia pulchra</i> ØSTRUP	1	-	-	- - 1 1 - -	- - - - -	- - - - -			
<i>Rhizosolenia eriensis</i> H.L.SMITH	1	1	1	- - - 1 - -	- - - 1 - 1	1 1 - 1 - -			
<i>Rhizosolenia longiseta</i> ZACH.	2	1	-	2 1 - - - -	1 - - 1 - -	- - - - -			
<i>Rhoicosphaenia abbreviata</i> (C.AG.)L-BERT.	1	1	2	1 3 1 1 1 2	- - 1 - 1 1	1 1 - 1 1 1			
<i>Rhopalodia gibba</i> (EHR.)O.MÜLL.	1	1	1	- - - 1 - -	- - - 1 - -	- 1 - 1 1 1			
<i>Rhopalodia gibba</i> var. <i>ventricosa</i> (EHR.)GRUN.	1	-	1	- - - - 1 -	- - - - -	- - - - 1 -			
<i>Skeletonema potamos</i> (WEBER)HASLE	-	1	1	- - - - -	1 3 1 1 1 1	1 1 1 1 1 -			
<i>Stauroneis phoenicenteron</i> (NITZSCH)EHR.	-	-	1	- - - - -	- - - - -	- - - - 1 -			
<i>Stauroneis smithii</i> GRUN.	1	-	-	- - - - 1 -	- - - - -	- - - - -			
<i>Stephanodiscus binderanus</i> (KÜTZ.)KRIEGER	-	-	1	- - - - -	- - - - -	1 1 1 1 1 -			
<i>Stephanodiscus hantzschii</i> GRUN.	2	1	1	2 2 2 2 - -	- - - 3 3 -	1 2 1 1 1 -			
<i>Stephanodiscus hantzschii</i> f. <i>tenuis</i> (HUST.)HÁK. et STOER.	3	1	2	2 2 2 2 - -	1 1 1 3 3 -	4 2 3 1 2 1			
<i>Stephanodiscus invisitatus</i> HOHN et HELLER	1	1	1	- - - - 1 -	1 1 1 1 1 -	- - 3 - - 1			
<i>Stephanodiscus minutulus</i> (KÜTZ.)CL. et MÜLL.	4	4	5	4 4 4 4 4 4	4 4 4 4 4 4	4 4 4 4 4 4			
<i>Surirella biseriata</i> var. <i>bifrons</i> (EHR.)HUST.	1	-	1	1 1 - - - -	- - - - -	1 - 1 - 1 1			
<i>Surirella biseriata</i> var. <i>bifrons</i> f. <i>punctata</i> MEISTER	2	1	1	1 1 1 1 1 1	- 1 1 - 1 -	1 1 1 1 - -			
<i>Surirella caproni</i> BRÉB.	-	-	1	- - - - -	- - - - -	1 - - - -			
<i>Surirella linearis</i> W.SM.	-	1	1	- - - - -	- - - 1 - -	1 - - - - 1			
<i>Surirella ovalis</i> BRÉB.	-	-	1	- - - - -	- - - - -	- - - - - 1			
<i>Surirella ovata</i> KÜTZ.	1	1	2	- - - - - 1	- 1 1 1 1 1	1 1 1 1 1 1			
<i>Surirella ovata</i> var. <i>crumena</i> (BRÉB.)V.H.	-	-	1	- - - - -	- - - - -	- - - 1 - -			
<i>Surirella ovata</i> var. <i>pinnata</i> (W.SM.)HUST.	-	-	1	- - - - -	- - - - -	- - - 1 - -			
<i>Surirella ovata</i> var. <i>pseudopinnata</i> - MAYER	2	1	1	1 1 1 1 1 1	1 - 1 1 - -	1 1 1 1 - 1			
<i>Surirella robusta</i> var. <i>splendida</i> (EHR.)V.H.	1	1	1	- 1 - - - -	1 - - 1 - -	- - 1 1 1 1			
<i>Surirella turgida</i> W.SM.	-	-	1	- - - - -	- - - - -	1 1 1 1 - -			
<i>Synedra acus</i> KÜTZ.	2	3	1	2 2 1 2 2 2	2 2 1 1 1 1	2 1 1 2 - 2			
<i>Synedra acus</i> var. <i>angustissima</i> GRUN.	1	2	1	1 1 2 2 1 1	1 1 1 1 1 1	2 1 - - 1 -			
<i>Synedra acus</i> var. <i>radians</i> (KÜTZ.)HUST.	1	2	2	1 4 4 - - 1	2 2 1 3 1 2	4 4 4 4 4 3			
<i>Synedra capitata</i> EHR.	1	-	1	- - - 1 1 -	- - - - -	- - - 1 1 -			

Table 1 (cont.)

Taxa	Constancy			Highest relative frequency					
	1983	1984	1985	1983	1984	1985	Sites of sampling		
				1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.			
<u>Synedra parasitica</u> var. <u>subconstricta</u> GRUN.	-	1	-	- - - - -	- 1 - - - -	- - - - -			
<u>Synedra tabulata</u> (AG.)KÜTZ.	-	1	-	- - - - -	1 - 1 1 1 1	- - - - -			
<u>Synedra tenera</u> W.SM.	1	-	-	- 1 1 - - -	- - - - -	- - - - -			
<u>Synedra ulna</u> (NITZSCH)EHR.	1	1	1	1 1 1 1 - -	1 1 1 1 1 1	1 1 - 1 1 1			
<u>Synedra ulna</u> var. <u>spathulifera</u> GRUN.	1	1	1	- - - 1 - -	- 1 - - - -	- 1 - 1 1 -			
<u>Tabellaria fenestrata</u> (LYNGB.)KÜTZ.	-	-	1	- - - - -	- - - - -	1 - - - -			
<u>Tabellaria flocculosa</u> (ROTH)KNUD.	-	-	1	- - - - -	- - - - -	1 - - - -			
<u>Thalassiosira weisflogii</u> (GRUN.)FRYXELL et HASLE	-	1	1	- - - - -	- - 1 1 - -	- 1 - - - 1			
PYRROPHYTA									
<u>Ceratium hirundinella</u> (O.F.MÜLL.)SCHRANK	2	1	2	2 2 2 2 2 4	1 1 1 - 1 2	1 1 1 1 1 1			
<u>Cryptomonas marssonii</u> SKUJA	2	1	1	3 3 3 2 2 4	- 1 - 1 1 -	- - 1 1 1 1			
<u>Cryptomonas ovata</u> EHR.	1	1	1	1 2 2 2 2 -	- - 1 - - -	- - 1 - - -			
<u>Cryptomonas pyrenoidifera</u> GEITL.	1	1	1	1 2 2 2 2 2	- - 1 - - -	1 1 - 1 1 1			
<u>Diplopsalis acuta</u> ENTZ	-	1	-	- - - - -	- - 1 1 - -	- - - - -			
<u>Peridinium aciculiferum</u> LEMM.	-	-	1	- - - - -	- - - - -	- - 1 1 1 1			
<u>Peridinium cinctum</u> (O.F.MÜLL.)EHR.	-	-	1	- - - - -	- - - - -	- - 1 1 1 1			
<u>Peridinium inconspicuum</u> LEMM.	-	1	1	- - - - -	1 4 3 1 2 -	1 1 1 - 1 1			
CHLOROPHYTA									
Volvocales									
<u>Pandorina morum</u> (MÜLL.)BORY	-	-	1	- - - - -	- - - - -	1 - - - -			
<u>Phacotus lenticularis</u> EHR.	4	2	4	3 2 2 3 3 2	1 1 1 1 1 1	2 2 1 1 1 1			
Chlorococcales									
<u>Actinastrum hantzschii</u> LAGH.	1	1	2	- 1 1 - 1 1	1 - - - -	1 1 1 1 1 1			
<u>Actinastrum subtile</u> WOLOSZ.	-	1	1	- - - - -	- - - 1 1	- - 1 - 1 1			
<u>Ankistrodesmus gracilis</u> (REINSCH)KORS.	-	1	-	- - - - -	1 - - - -	- - - - -			
<u>Ankyra judayi</u> (G.M.SM.)FOTT	1	-	1	- - - - 1	- - - - -	1 1 - - -			
<u>Ankyra lanceolata</u> (KORS.)FOTT	-	-	1	- - - - -	- - - - -	- 1 1 1 -			
<u>Ankyra ocellata</u> (KORS.)FOTT	-	1	-	- - - - -	1 - - 1 1 1	- - - - -			
<u>Botryococcus braunii</u> KÜTZ.	1	-	-	1 - - 1 - -	- - - - -	- - - - -			
+ <u>Chodatellopsis elliptica</u> KORS.	-	1	-	- - - - -	- - - 1 - -	- - - - -			
<u>Coelastrum microporum</u> NAEG. in A.BR.	2	1	1	1 1 2 1 1 1	- - - - 1 -	- - 1 1 1 1			
<u>Coelastrum proboscideum</u> BOHL. in WITTR. et NORDST.	-	1	1	- - - - -	1 1 1 1 1 1	1 1 1 1 - 1			
<u>Coelastrum pseudomicroporum</u> KORS.	1	1	1	1 1 1 1 1 -	1 - - - -	1 1 1 1 1 1			
<u>Coelastrum sphaericum</u> NAEG.	1	1	1	1 1 1 2 1 -	1 1 1 1 - -	1 1 1 1 1 1			
+ <u>Coronastrum ellipsoideum</u> FOTT	1	-	-	- 1 - - -	- - - - -	- - - - -			
<u>Crucigenia smithii</u> (BOURR. et MANGUIN)KOM.	1	2	1	- 2 1 1 2 1	1 1 1 1 1 1	1 1 - 1 1 1			

Table 1 (cont.)

Taxa	Constancy			Highest relative frequency					
	1983	1984	1985	1983	1984	1985	Sites of sampling		
				1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.
<i>Crucigenia quadrata</i> MORREN	2	4	3	2 2 2 2 3 1	1 1 1 1 2 1	1 1 1 1 1 1			
<i>Crucigenia tetrapedia</i> (KIRCHEN.)W. et G.S. WEST	1	1	-	1 1 - - - -	- - 1 1 - -	- - - - - -			
<i>Crucigeniella apiculata</i> (LEMM.)KOM.	1	-	1	- - 1 1 1 -	- - - - - -	- - - - 1 -			
<i>Crucigeniella pulchra</i> (W. et G.S. WEST)KOM.	1	1	-	2 2 - - 1 1	- - - 1 - -	- - - - - -			
<i>Dictyosphaerium anomalum</i> KORS.	1	-	-	1 - 1 - - -	- - - - - -	- - - - - -			
<i>Dictyosphaerium ehrenbergianum</i> NAEG.	2	1	-	1 2 2 2 - 1	- - - - - -	1 1 1 - 1 1			
<i>Dictyosphaerium pulchellum</i> WOOD	4	4	3	2 2 2 2 2 3	1 2 2 1 1 2	1 1 1 1 1 1			
<i>Didymogenes palatina</i> SCHMIDLE	1	1	1	- - - 1 - -	1 1 - - - 1	1 - - - - -			
<i>Dimorphococcus lunatus</i> A.BR.	1	-	-	- - 1 - - -	- - - - - -	- - - - - -			
<i>Elakathotrix lacustris</i> KORS.	1	2	2	1 1 - 1 1 -	1 1 1 1 1 1	1 1 1 1 1 1			
<i>Elakathotrix subacuta</i> KORS.	1	-	-	- - 1 - - -	- - - - - -	- - - - - -			
<i>Eutetramos planctonicus</i> (KORS.) BOURR.	1	-	-	1 1 2 2 2 2	- - - - - -	- - - - - -			
<i>Franceia ovalis</i> (FRANCE)LEMM.	-	-	1	- - - - - -	- - - - - -	- 1 - - - -			
<i>Golenkinia radiata</i> CHOD.	1	1	1	1 1 - - 1 -	- 1 1 1 1 -	- - - - - 1			
<i>Golenkiniopsis longispina</i> KORS.	-	1	-	- - - - - -	- - 1 1 - -	- - - - - -			
<i>Golenkiniopsis solitaria</i> KORS.	-	1	-	- - - - - -	- - - 1 - -	- - - - - -			
<i>Kirchneriella irregularis</i> (G.M.SM.)KORS.	1	1	1	- 1 - - 1 -	1 - - - - -	- - - 1 - -			
<i>Kirchneriella lunaris</i> (KIRCHN.)MOEB.	1	1	-	- 1 - - - -	- - - - - 1	- - - - - -			
<i>Kirchneriella obesa</i> (W.WEST.) SCHMIDLE	1	1	1	1 - - - - -	- - - - - 1	1 - - - - -			
<i>Lagerheimia balatonica</i> (SCHERFF. in KOL)HIND.	1	1	1	- 1 1 1 1 -	- 1 1 1 - 1	1 1 1 - - -			
<i>Lagerheimia cingula</i> G.M.SMITH	1	-	-	- 1 - - - -	- - - - - -	- - - - - -			
<i>Lagerheimia genevensis</i> (CHOD.)CHOD.	1	1	-	- - 1 1 2 1	1 1 1 1 1 1	1 - 1 1 1 1			
<i>Lagerheimia longiseta</i> LEMM.	-	1	-	- - - - - -	- - - - 1 -	- - - - - -			
<i>Lagerheimia subsalsa</i> LEMM.	1	1	1	1 - - - 1 -	1 1 1 1 1 1	- - 1 - 1 -			
<i>Lagerheimia wratislawiensis</i> SCHRÖD.	1	1	-	- - - 1 - -	1 1 - 1 1 -	- - - - - -			
<i>Micratinium crassisetum</i> HORTOB.	1	-	-	- 1 - - - -	- - - - - -	- - - - - -			
<i>Micratinium pusillum</i> FRESEN	1	1	1	1 1 1 - - -	- - - 1 - -	1 1 1 1 1 1			
<i>Monoraphidium contortum</i> (THUR.) KOM.-LEGN.	2	4	2	2 2 2 2 2 2	3 3 3 3 4 -	1 1 1 1 1 1			
<i>Monoraphidium griffithii</i> (BERK.)KOM.-LEGN.	1	2	2	1 1 1 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1			
<i>Monoraphidium minutum</i> (NAEG.) KOM.-LEGN.	-	1	1	- - - - - -	- - - - 1 -	1 1 - - - -			
<i>Nephroclamys subsolitaria</i> (G.S.WEST)KORS.	1	1	1	1 1 1 - 1 -	- 1 1 - 1 1	- 1 - 1 1 -			
<i>Oocystis cingulata</i> HORTOB. et NÉMETH	1	-	-	- - - 1 - 1	- - - - - -	- - - - - -			
<i>Oocystis marssonii</i> LEMM.	1	-	-	2 1 2 1 1 1	- - - - - -	- - - - - -			
<i>Oocystis natans</i> (LEMM.)LEMM.	1	-	1	1 1 1 1 1 2	- - - - - -	1 1 - - - -			
<i>Oocystis parva</i> W. et G.S.WEST	4	2	1	3 3 2 3 2 2	1 1 1 1 1 2	- 1 1 - - -			
<i>Pediastrum boryanum</i> (TURP.)MENECH.	2	2	2	1 1 1 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1			

Table 1 (cont.)

Taxa	Constancy			Highest relative frequency					
	1983	1984	1985	1983	1984	1985	Sites of sampling		
				1.2.3.4.5.6.			1.2.3.4.5.6.	1.2.3.4.5.6.	1.2.3.4.5.6.
<u>Pediastrum duplex</u> MEYEN	2	1	2	1 1 1 1 1 2	1 1 1 - 1 1	1 1 1 1 1 1			
<u>Pediastrum duplex</u> var. <u>gracillimum</u> W. et G.S.WEST	1	-	-	- 1 - - - -	- - - - - -	- - - - - -			
<u>Pediastrum simplex</u> MEYEN	1	1	1	1 1 1 - 1 1	1 - 1 1 - -	- - 1 1 1 1			
<u>Pediastrum simplex</u> var. <u>echinulatum</u> (WITTR.) et NORDST.	1	-	-	1 1 1 - - -	- - - - - -	- - - - - -			
<u>Pediastrum tetras</u> (EHR.)RALFS	1	2	2	1 1 1 1 1 1	1 1 1 1 - -	1 1 1 1 1 1			
<u>Pediastrum tetras</u> var. <u>tetraodon</u> f. <u>globosum</u> HORTOB.	1	1	1	1 1 1 - 1 -	1 - 1 1 1 -	- - 1 1 1 -			
<u>Radiococcus wildemani</u> SCHMIDLE	1	-	-	2 - - - - 1	- - - - - -	- - - - - -			
<u>Scenedesmus aculeolatus</u> REINSCH	1	1	1	- - - 1 - -	1 - - - - -	1 - 1 1 - 1			
<u>Scenedesmus acuminatus</u> (LACH.)CHOD.	1	1	1	1 2 1 1 2 1	2 1 1 - - -	1 1 1 1 1 1			
<u>Scenedesmus acutus</u> MEYEN	1	1	1	1 1 1 1 - 1	1 1 1 1 1 1	1 1 1 1 1 1			
<u>Scenedesmus antennatus</u> BRÉB. in RALFS	1	1	1	1 1 1 - 2 -	1 1 1 1 1 1	1 1 1 1 1 1			
<u>Scenedesmus balatonicus</u> HORTOB.	1	2	3	1 1 - 1 - -	1 1 1 - 1 1	1 1 1 1 1 1			
<u>Scenedesmus carinatus</u> (LEMM.)CHOD.	-	1	1	- - - - - -	- - - - 1 1	- - - - 1 -			
<u>Scenedesmus columnatus</u> HORTOB.	1	-	-	- 1 - - - -	- - - - - -	- - - - - -			
<u>Scenedesmus decorus</u> HORTOB.	1	-	-	1 - - - - -	- - - - - -	- - - - - -			
<u>Scenedesmus disciformis</u> (CHOD.) FOTT f. <u>disciformis</u>	1	1	1	1 1 1 - - -	- - - - 1 1	- - - - - 1			
<u>Scenedesmus granulatus</u> W. et G.S.WEST	1	1	1	1 1 - - - -	1 1 1 1 1 1	1 1 1 1 1 1			
<u>Scenedesmus gutwinski</u> CHOD.	1	1	-	1 - - - - -	- - 1 - - -	- - - - - -			
<u>Scenedesmus intermedius</u> CHOD.	2	2	1	1 1 2 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1			
<u>Scenedesmus intermedius</u> var. <u>acaudatus</u> HORTOB.	1	1	1	- - - - 1 1	1 - - - - 1	1 - - - - -			
<u>Scenedesmus longispina</u> CHOD.	1	1	-	1 1 1 1 - -	1 - - - - 1	- - - - - -			
<u>Scenedesmus obtusus</u> MEYEN f. <u>obtus</u>	1	-	-	1 - - - - -	- - - - - -	- - - - - -			
<u>Scenedesmus opoliensis</u> P.RICHT.	1	1	1	1 1 - 1 1 1	1 1 1 - 1 -	1 1 1 - 1 1			
<u>Scenedesmus quadricauda</u> (TURP.)BRÉB.	2	2	1	2 2 2 - 1 1	1 1 1 1 1 1	1 1 1 1 - -			
<u>Scenedesmus spinosus</u> CHOD.	4	4	4	2 2 2 2 2 1	1 1 2 1 1 1	1 1 1 1 1 1			
<u>Schroederia setigera</u> (SCHRÖD.)LEMM.	1	2	1	1 - 1 3 3 -	1 1 1 1 - 1	1 1 - 1 - -			
<u>Schroederia spiralis</u> (PRINTZ)KORS.	-	1	-	- - - - - -	- - 1 - - -	- - - - - -			
<u>Tetraedron caudatum</u> (CORDA)HANGS.	2	3	3	1 2 - 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1			
<u>Tetraedron incus</u> (TEIL.)G.M.SM.	-	1	-	- - - - - -	- 1 - - - -	- - - - - -			
<u>Tetraedron minimum</u> (A.BR.)HANGS.	-	2	3	- - - - - -	1 1 1 1 1 1	1 1 1 1 1 1			
<u>Tetrastrum elegans</u> PLAYF.	1	2	3	1 1 2 1 1 1	1 1 1 1 1 1	1 1 1 1 1 1			
<u>Tetrastrum glabrum</u> (ROLL)AHISTR. et TIFF.	-	1	-	1 1 1 - - -	1 1 1 - 1 -	- - - - - -			
<u>Tetrastrum staurogeniaeforme</u> (SCHRÖD.)LEMM.	2	3	3	2 2 2 2 1 1	1 1 1 1 1 1	1 1 1 1 1 1			
<u>Treubaria schmidlei</u> (SCHRÖD.)FOTT	1	-	1	1 - - - - -	- - - - - -	- - - 1 - -			
<u>Treubaria setigera</u> (ARCH)G.M.SM.	1	-	-	- - - - - 1	- - - - - -	- - - - - -			
<u>Treubaria triappendiculata</u> BERN.	1	1	1	1 1 - 1 1 -	1 1 - 1 - -	1 1 1 - 1 1			
<u>Westella botryoides</u> (W.WEST) DE WILD	1	-	-	- 1 - 1 - -	- - - - - -	- - - - - -			

Table 1 (cont.)

Taxa	Constancy			Highest relative frequency																	
	1983	1984	1985	1983						1984						1985					
	Sites of sampling 1.2.3.4.5.6. 1.2.3.4.5.6. 1.2.3.4.5.6.																				
Ulotrichales																					
<u>Planctonema lauterbornii</u> SCHMIDLE		4	4	2	4	2	2	3	2	2	3	3	3	3	4	1	1	1	1	1	
Desmidiaceae																					
<u>Closterium aciculare</u> T.WEST.		1	1	2	2	2	-	-	1	1	-	-	-	-	-	1	1	1	1	1	
<u>Closterium acutum</u> BRÉB.		1	-	-	-	-	2	2	1	-	-	-	-	-	-	-	-	-	-	-	
<u>Closterium acutum</u> var. <u>variabile</u> (LEMM.)KRIEG.		1	1	1	-	-	1	-	1	1	1	1	-	-	-	1	1	1	1	1	
<u>Closterium limneticum</u> LEMM.		1	1	1	-	-	1	-	-	1	-	2	-	1	-	1	1	1	1	1	
<u>Cosmarium bioculatum</u> (BRÉB.)RALFS		1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<u>Cosmarium depressum</u> (NÆG.)LUND		1	2	2	1	1	-	-	1	1	-	1	1	1	1	1	1	1	1	1	
<u>Cosmarium humile</u> (GAY)NORDST.		1	1	1	1	1	-	-	1	-	-	-	-	1	1	-	1	1	1	1	
<u>Cosmarium margaritifera</u> (TURP.)MENECH.		1	1	1	1	1	-	-	-	-	-	1	-	-	-	-	-	1	-	-	
<u>Cosmarium ornatum</u> RALFS		1	-	-	1	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	
<u>Cosmarium phaseolus</u> var. <u>minus</u> (BOLDT)KRIEG.		1	1	1	1	-	1	1	1	1	1	1	-	1	-	1	1	1	1	-	
<u>Cosmarium praecisum</u> var. <u>suecicum</u> (BORGE)KRIEG. et GERL.		1	-	1	1	-	1	-	-	-	-	-	-	-	-	1	1	1	1	1	
<u>Staurastrum paradoxum</u> MEYEN		2	2	2	1	-	1	-	-	-	1	1	-	1	1	1	1	1	1	1	
<u>Teilingia granulata</u> (ROY et BISS.)BOURR.		1	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	

Signs in Table 1:

Initial letter underlined = taxa new compared to the author's 1982 data

* = occurrence new in Hungary

+ = occurrence new in Lake Balaton

Results

Qualitative analyses

Taxa found between 1983 and 1985 are contained in Table 1. The highest frequency characteristic of the individual sampling sites is denoted with 1-4 (1 = 1-3 algae, 2 = 3-20 algae, 3 = 20-40 algae, 4 = more than 40 algae in one drop of the homogenized sediment of 250 ml centrifuged water). Further, the table contains the values of constancy characteristic of the respective years at all sites of sampling. (Values of constancy:

- 1 = for the taxon occurring in 1- 19%
- 2 = for the taxon occurring in 20- 39%
- 3 = for the taxon occurring in 40- 59%
- 4 = for the taxon occurring in 60- 79%
- 5 = for the taxon occurring in 80-100%

of the total number of samples.)

Number of taxa found between 1983 and 1985, by taxonomic distribution:

Cyanophyta	46	Pyrrophyta	8
Euglenophyta	16	Volvocales	2
Xanthophyceae	1	Chlorococcales	88
Chrysophyceae	6	Desmidiales	13
Bacillariophyceae	225		

In the 198 samples collected in three years altogether 406 taxa were found. The list of species was compared with the alga catalogue "Flora et iconographia algarum Hungariae" in the Botanical Collection of the Hungarian National Museum, Department of Natural Sciences and with the latest publications connected with Hungary in order to establish the taxa new for Hungary and for Lake Balaton (HORTOBÁGYI 1974).

Species new in the flora of Hungary are: Aphanothece stagnina (Cyanophyta), Amphora thumensis, Epithemia mülleri, Fragilaria construens var. triundulata, Gyrosigma prolongatum var. closteroides, Navicula platystoma, Nitzschia palaeformis, Oestrupia bicontracta, Pinnularia braunii var. amphycephala, P. lata (Bacillariophyceae), Lagerheimia cingula (Chlorococcales). In the algal flora of Lake Balaton new species are: Amphora commutata, Bacillaria paradoxa var. tumidula, Navicula exigua, N. kotschyi, Nitzschia dubia, Pinnularia pulchra (Bacillariophyceae), Chodatellopsis elliptica, Coronastrum ellipaoideum, Elakathotrix subacuta, Eutetramos planctonicus, Golenkiniopsis solitaria (Chlorococcales).

Number of taxa in the successive years

	1983	1984	1985
Cyanophyta	31	31	22
Euglenophyta	12	8	11
Xanthophyceae	1	—	—
Chrysophyceae	2	4	4
Bacillariophyceae	128	139	182
Pyrrophyta	4	6	7
Volvocales	1	1	2
Chlorococcales	73	62	55
Ulotrichales	1	1	1
Desmidiales	13	8	9
Total:	266	260	293

The stability of the taxa is characterized by the values of constancy (see Table 1). Percentage distribution of taxa on the basis of the values of constancy:

Constancy	1983	1984	1985
1	76.8%	70.0%	72.8%
2	14.0%	16.9%	15.9%
3	4.0%	6.6%	5.6%
4	5.2%	3.7%	4.0%
5	0.0%	2.8%	1.7%

Quantitative analyses

The minima and maxima of the number of algae per litre are contained in Table 2. In 1983 the largest number of algae ($47 \cdot 10^6 \cdot \text{dm}^{-3}$) was found on the Helikon beach at Keszthely in May. In 1984 the number of algae gave a maximum value ($62.5 \cdot 10^6 \cdot \text{dm}^{-3}$) in August at Balatonberény, and this was the place for the 1985 maximum too ($41.4 \cdot 10^6 \cdot \text{dm}^{-3}$) in May. In 1986 the largest number of algae were found at Balatongyörök in August ($80.3 \cdot 10^6 \cdot \text{dm}^{-3}$). In 1987 the maximum was recorded again at Balatonberény in September ($36.4 \cdot 10^6 \cdot \text{dm}^{-3}$).

The composition and succession of the phytoplankton are shown in Figs 2--3. The spring and the summer period are rather different. In spring diatoms represented 53-60% in 1983, 47-98% in 1984, 33-76% in 1985, 68-92% in 1986 and 23-59% in 1987 of the total number of algae. In summer the ratio changed in favour of the blue algae which in 1983 made 38-63%, in 1984 89-95%, in 1985 72-91%, in 1986 70-88% and in 1987 51-81% of the total number of algae.

The minima and maxima of the chlorophyll-a concentration, an indicator of the extent of trophity, are also seen in Table 2. Between 1983 and 1986 the annual maxima exceeded $100 \text{ mg} \cdot \text{m}^{-3}$, while in 1987 they were below this value. Outstandingly high concentration was measured in 1986 ($230 \text{ mg} \cdot \text{m}^{-3}$) on the Gyenesdiás beach. In that year the maximum concentration was above $100 \text{ mg} \cdot \text{m}^{-3}$ at all sites of sampling.

Annual average chlorophyll-a concentrations ($\text{mg} \cdot \text{m}^{-3}$):

Site	Time					
	1982	1983	1984	1985	1986	1987
1.	66.1	30.0	54.6	41.5	59.6	29.6
2.	81.0	28.7	56.8	34.8	67.0	32.5
3.	86.3	32.2	58.1	37.7	68.4	32.8
4.	62.5	26.1	44.0	42.7	63.2	26.3
5.	70.2	24.9	42.5	42.6	62.0	27.6
6.	51.8	16.0	32.6	25.4	48.8	21.8

Table 2
Chlorophyll-a ($\text{mg}\cdot\text{m}^{-3}$) and total alga ($10^6\cdot\text{dm}^{-3}$) maxima on beaches in the Keszthely Bay

	1983				1984				1985				1986				1987			
	Chlor.-a		Total alga		Chlor.-a		Total alga		Chlor.-a		Total alga		Chlor.-a		Total alga		Chlor.-a		Total alga	
	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
1.	12.3	106.5	8.6	39.0	23.6	98.3	4.8	62.5	10.6	84.2	6.8	41.4	8.4	141.0	7.8	73.8	6.8	62.0	5.53	36.35
2.	14.5	81.2	8.0	47.3	15.3	111.7	6.0	49.4	7.6	76.6	3.0	36.1	8.8	155.4	5.9	69.4	7.2	71.2	3.4	29.0
3.	16.2	92.1	3.5	41.0	14.3	117.3	0.48	46.2	10.0	91.2	3.6	39.8	10.4	174.5	8.5	67.0	7.2	72.3	5.78	24.1
4.	12.1	52.8	1.8	26.7	14.9	104.2	2.4	42.7	9.2	161.0	3.2	30.5	10.6	230.0	7.5	66.3	7.0	59.0	3.6	21.7
5.	11.7	84.0	2.1	26.3	13.0	96.2	4.6	38.7	12.4	75.9	6.1	41.3	5.6	213.0	5.8	62.8	10.3	58.5	4.0	21.7
6.	5.6	37.6	1.2	22.8	7.2	86.7	1.7	36.9	11.2	49.4	3.1	20.7	4.0	153.0	2.8	80.3	9.0	36.5	2.0	36.5

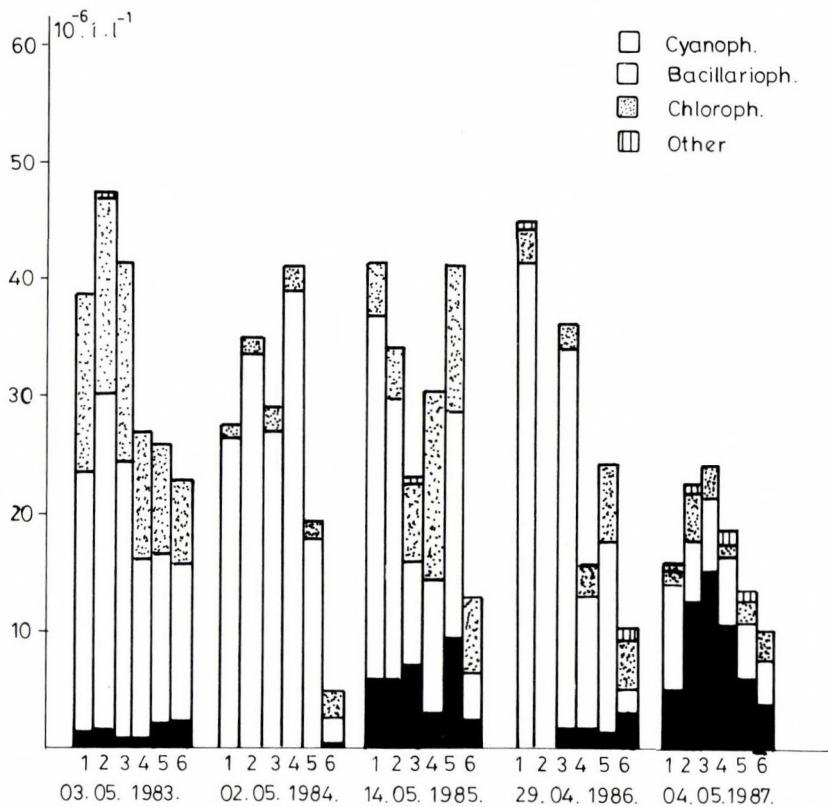


Fig. 2. Composition of phytoplankton in spring
(data of some characteristic sample series)

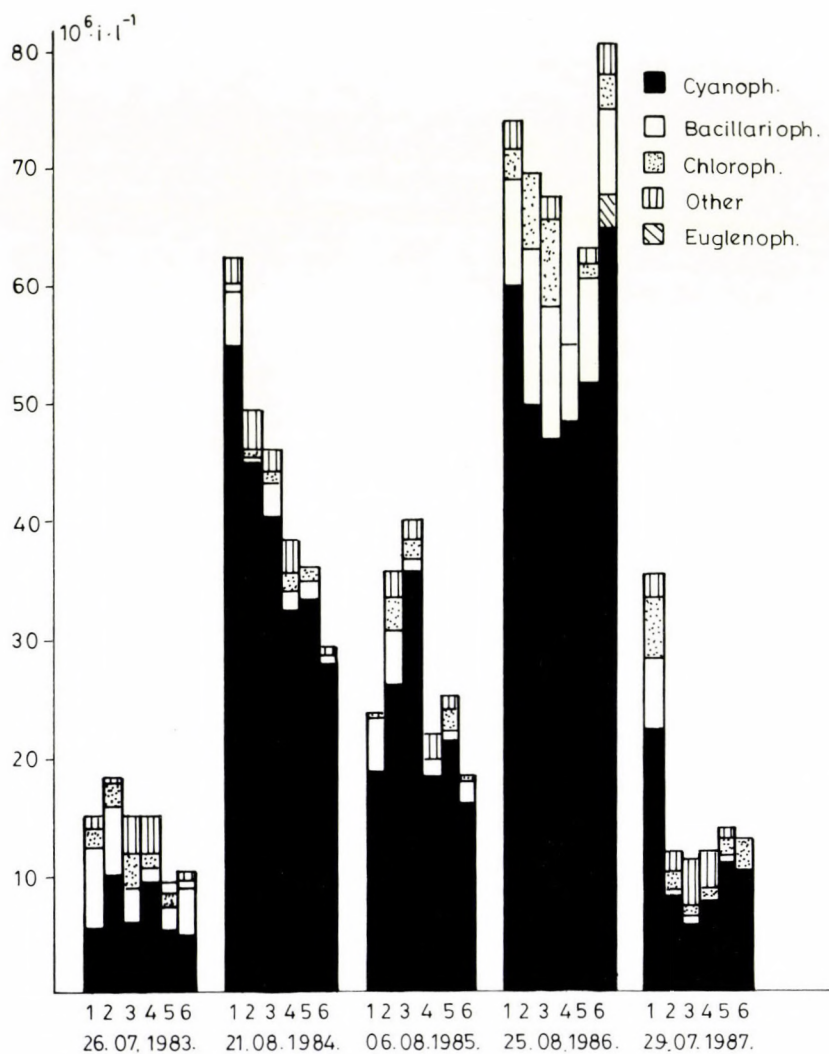


Fig. 3. Composition of phytoplankton in summer
(data of some characteristic sample series)

Discussion

As regards the number of taxa, in the composition of the phytoplankton the diatoms were dominant, followed by the blue algae from the order of Chlorococcales. Out of the diatoms the genera Nitzschia and Navicula, while among the blue algae the Scenedesmus genus were the richest in species. Beside the euplanktonic species there are many tichoplanktonic elements in the phytoplankton, a characteristic feature of Lake Balaton which is often disturbed by storms (PADISÁK et al. 1988; TAMÁS 1969, 1972; VÍZKELETY 1987—1988).

Such members of the phytoplankton as new for Hungary are: 1 blue alga, 9 diatoms, 1 green alga; those new for Lake Balaton: 6 diatom and 5 green alga taxa. In Table 1 the taxa new for Hungary are marked with asterisk, those new for Lake Balaton with +, while the taxa new in comparison to our 1982 list of species are indicated by underlining the initial letter. Compared to the 1982 occurrence 129 new taxa were found between 1983 and 1985 (17 Cyanophyta, 10 Euglenophyta, 1 Xanthophyceae, 3 Chrysophyceae, 61 Bacillariophyceae, 3 Pyrrophyta, 27 Chlorococcales, 7 Desmidiaceae). Taxa occurring in masses on all beaches between 1983 and 1985 are: Anabaenopsis raciborskii, Aphanizomenon flos-aquae f. kelbahnii (Cyanophyta), Nitzschia acicularis, Stephanodiscus minutulus (Bacillariophyceae).

Taxa occurring in masses only in certain years: Anabaena aphanizomenoides, A. spiroides, Lyngbya limnetica, Microcystis minutissima (Cyanophyta), Asterionella formosa, Cyclotella comta, Cyclostephanos dubius, Melosira granulata var. angustissima, Nitzschia actinastroides (Bacillariophyceae).

The highest constancy taxa made 1-5 per cent of the total. The species of constancy 4 and 5 in Table 1 are the stable algae of the Keszthely Bay (Anabaenopsis raciborskii, Aphanizomenon flos-aquae f. kelbahnii, Gomphosphaeria lacustris, Lyngbya limnetica, Microcystis minutissima, Melosira granulata var. angustissima, Nitzschia acicularis, Scenedesmus spinosus). Most of the taxa were of constancy 1 (77% in 1983, 70% in 1984, 73% in 1985). Similar results were obtained by PADISÁK et al. (1988) during investigations in other parts of Lake Balaton. With the absence or presence of taxa in Table 1 taken into consideration it can be established that 36-59% of the Cyanophytes, 22-25% of the Bacillariophyceae and 22-49% of the Chlorococcales occurred on all beaches. Of the total number of taxa in 1983 25%, in 1984 26% and in 1985 28% were present on all beaches.

Table 4
Dominant taxa in 1984

	05.02.	05.15.	05.29.	06.12.	06.26.	07.10.	07.24.	08.07.	08.21.	09.04.	09.18.
<u>Anabaenopsis raciborskii</u>	-	-	-	-	-	-	-	+	+	+	+
<u>Ankistrodesmus angustus</u>	+	-	-	-	-	-	-	-	-	-	-
<u>Aphanizomenon flos-a. f. klebahnii</u>	-	-	-	-	-	+	+	+	-	-	-
<u>Cyclotella ocellata</u>	-	-	-	-	-	-	-	+	-	-	-
<u>Fragilária construens</u>	-	-	-	-	-	-	+	-	-	-	-
<u>Fragilaria inflata</u>	-	-	-	-	-	-	+	-	-	-	-
<u>Gomphonema lacustris</u>	-	-	-	-	-	+	-	-	-	-	-
<u>Gomphonema lacustris var. compacta</u>	-	-	-	-	-	+	-	-	+	-	-
<u>Lyngbya limnetica</u>	-	-	-	-	+	-	+	+	+	+	-
<u>Microcystis minutissima</u>	-	-	-	-	+	+	+	+	+	-	-
<u>Nitzschia acicularis</u>	+	+	+	+	+	-	-	+	+	+	+
<u>Nitzschia actinastroides</u>	-	+	+	+	-	+	-	+	-	-	+
<u>Planctonema lauterbornii</u>	-	-	-	-	+	+	-	-	-	-	-
<u>Scenedesmus spp.</u>	-	-	-	-	-	-	-	+	+	+	+
<u>Stephanodiscus minutulus</u>	+	+	+	+	+	+	-	-	-	-	-
<u>Synedra acus var. radians</u>	+	-	-	-	-	-	-	-	-	-	+

Table 5
Dominant taxa in 1985

	04.29.	05.14.	05.28.	06.11.	06.25.	07.09.	07.23.	08.06.	08.21.	09.03.	09.17.
<u>Anabaena aphanizomenoides</u>	-	-	-	-	-	-	+	-	-	-	-
<u>Anabaena spiroides</u>	-	-	-	-	-	-	+	-	-	-	-
<u>Anabaenopsis raciborskii</u>	-	-	-	-	-	-	+	+	+	+	+
<u>Aphanizomenon flos-a. f. klebahnii</u>	-	+	-	-	-	-	+	-	+	-	-
<u>Aphanizomenon issatschenkoi</u>	-	-	-	-	-	-	+	-	+	-	-
<u>Cyclostephanos dubius</u>	-	-	-	+	+	+	-	-	-	-	-
<u>Cyclotella comta</u>	-	+	+	+	+	+	-	-	-	-	-
<u>Cyclotella ocellata</u>	-	-	-	+	+	+	+	-	+	-	-
<u>Cyclotella pseudostelligera</u>	-	+	+	-	-	-	-	-	-	-	-
<u>Fragilaria conctruens</u>	-	+	-	-	-	-	-	-	-	-	-
<u>Fragilaria inflata</u>	-	+	-	-	-	-	-	-	-	-	-
<u>Gomphonema lacustris</u>	-	-	-	-	-	+	-	-	-	-	-
<u>Lyngbya limnetica</u>	+	+	-	-	-	-	-	+	-	-	-
<u>Nitzschia acicularis</u>	+	+	-	-	+	+	+	+	+	+	+
<u>Stephanodiscus hantzschii</u> var. <u>tenuis</u>	-	-	-	-	-	-	-	-	+	-	+
<u>Stephanodiscus minutulus</u>	+	+	+	+	+	+	+	-	+	+	+
<u>Synedra acus</u> var. <u>radians</u>	+	+	-	-	-	-	+	-	-	+	+

As regards the composition of the phytoplankton the Balatonberény beach differs from the others in the greatest measure, as more benthic algae are carried here into the phytoplankton by the waves (Fragilaria construens, F. inflata, F. pinnata).

According to the composition of the phytoplankton spring-, summer- and summer-early autumn periods can be distinguished, plus a transitional stage in June. In spring the diatoms (Cyclotella comta, C. ocellata, C. pseudostelligera, Stephanodiscus minutulus, Nitzschia acicularis, N. actinastroides, Synedra acus var. radians) became numerous. The time of dominant and subdominant occurrence is shown in Tables 3--5. As seen in the table, in spring -- at the time of the multiplication of diatoms -- Nitzschia acicularis, Stephanodiscus minutulus and Synedra acus var. radians were dominant in all three years (1983--1985), while the other species -- Centricae, the taxon characteristic of Lake Balaton -- multiplied remarkably only one or the other year. In 1983 Asterionella formosa, Cyclostephanos dubius, in 1985 Cyclotella comta, C. ocellata, C. pseudostelligera were dominant in spring, while in summer Asterionella formosa, Melosira granulata var. angustissima were the dominant diatoms. TAMÁS (1966) described a discoloration of water caused by Melosira granulata var. angustissima and M. granulata in 1965. KISS and PADISÁK (1988) experienced a similar seasonality in the Keszthely Bay in 1980.

In summer the diatoms decreased in number by July and the blue algae (Anabaena aphanizomenoides, A. spiroides, Anabaenopsis raciborskii, Aphanizomenon flos-aquae f. klebahnii, A. issatschenkoi) became numerous. This phenomenon is characteristic of shallow waters abounding in nutrients. TAMÁS (1974) observed Aphanizomenon flos-aquae bloom in the Keszthely Bay in 1966 already. VÖRÖS (1980) also described the mass reproduction of Aphanizomenon flos-aquae, Anabaena spiroides in 1976. VÖRÖS et al. (1983) reported the summer dominance of Anabaenopsis raciborskii in the Keszthely Bay in 1979. VÍZKELETY (1987--88), G. TÓTH and PADISÁK (1986) observed in 1982 the so far greatest multiplication of the blue alga Anabaenopsis raciborskii over the entire area of Lake Balaton.

The years 1985 and 1987 were somewhat different, because in these years the blue algae (Aphanoteca clathrata, Lyngbya limnetica, Aphanizomenon flos-aquae f. klebahnii, Microcystis minutissima) already appeared in spring. According to VÖRÖS (1987--1988) the algae mentioned as Microcystis minutissima belonged to the genera Synechococcus and Synechocystis. Out of the blue algae Anabaenopsis raciborskii prevailed for the longest time, up

Table 6

Chlorophyll-a concentration maxima

Year	Conc. ($\text{mg}\cdot\text{m}^{-3}$)	Site
1975.	110.0	Balatonberény shore
1976.	43.0	Mouth of the river Zala
1979.	130.0	Keszthely-mouth of Bűdös árok
1982.	244.0	Keszthely-City Beach
1983.	106.0	Balatonberény beach
1984.	117.3	Keszthely-City Beach
1985.	161.0	Gyenesdiás beach
1986.	230.0	Gyenesdiás beach
1987.	72.3	Keszthely-City Beach

Chlorophyll-a concentration averages ($\text{mg}\cdot\text{m}^{-3}$)

Site	Time					
	1982	1983	1984	1985	1986	1987
1.	66.1	30.0	54.6	41.5	59.6	29.6
2.	81.0	28.7	56.8	34.8	67.0	32.5
3.	86.3	32.3	58.1	37.7	68.4	32.8
4.	62.5	26.1	44.0	42.7	63.2	26.3
5.	70.2	24.9	42.5	42.6	62.0	27.6
6.	51.8	16.0	32.6	25.4	48.8	21.8

to the end of September, and beside it Nitzschia acicularis multiplied again every year.

The maximum of the total alga number was everywhere high (1983: $47.10^6\cdot\text{dm}^{-3}$, 1984: $62.5\cdot 10^6\cdot\text{dm}^{-3}$, 1985: $41.4\cdot 10^6\cdot\text{dm}^{-3}$, 1986: $80.3\cdot 10^6\cdot\text{dm}^{-3}$, 1987: $24.1\cdot 10^6\cdot\text{dm}^{-3}$). On the Balatonyörök beach in 1986 the maximum came close to the so far largest quantity registered in 1982.

As regards the chlorophyll-a concentration the maximum was the highest in 1986, equal to the 1982 value. On the basis of the average concentration of chlorophyll-a 1986 was the most eutrophic year, followed by 1984, 1985, 1987 and 1983. According to FELFÖLDY (1980) all beaches were mesoeutrophic in 1983–1985, and eutrophic in 1986 with the exception of the Balatonyörök beach which proved invariably meso-eutrophic. In 1987 the water was meso-eutrophic all over the Keszthely Bay. In general, the chlorophyll-a concentration was highest on the beaches of Keszthely and Gyenesdiás. A comparison of the annual maxima to earlier data (Table 6) reveals that the maximum concentration in the Bay was already above $100\text{ mg}\cdot\text{m}^{-3}$ in 1975. 1982 and 1986

were years with extreme algal bloom, while 1976 and 1987 were the most favourable years from the point of view of eutrophy.

To sum it all up we can say that on the beaches (littoral zone) of the Keszthely Bay the composition of phytoplankton is highly diversified. Differences between the beaches are due to the frequent storms and to the different conditions of waves. Among the taxa there are many algae brought up from the sediment, particularly on the Balatonberény beach. The planktonic algae are more uniformly distributed, the dominant taxa are identical everywhere. The algal composition changes several times from spring to autumn. The dominance of diatoms in spring, of blue algae in summer and of blue algae-diatoms in autumn is characteristic. The green algae are rich in species and accompany the former. In comparison to the 1970s an increasing number of blue alga taxa appear in masses in the 1980s. The trophity level of the Bay was high even in the seventies. In the eighties eutrophication was outstandingly high in two years, characterized by an extensive bloom of blue algae. Comparing the beaches for trophity level we find a decreasing tendency from the Keszthely beaches eastwards up to 1985, while in 1986 the trophity was already uniform.

REFERENCES

For the studies and articles cited in the text see:

Vízkelety, É. (1987--1988): Adatok a Keszthelyi-öböl algológiai és trofitási viszonyaihoz (Data to the algological and trophical relations of the Keszthely Bay). Bot. Közl. 74—75: 153-192.

Plate I

Fig. 1. Cyclotella meneghiniana

Fig. 2. Cyclotella pseudostelligera

Fig. 3. Cyclotella comta

Fig. 4. Fragilaria inflata

Fig. 5. Cymbella affinis

Fig. 6. Cocconeis placentula

Fig. 7. Cyclotella ocellata

Fig. 8. Gomphonema parvulum var. micropus

Fig. 9. Epithemia zebra var. saxonica

Plate II

Fig. 10. Diatoma vulgare var. productum

Fig. 11. Navicula tripunctata

Fig. 12. Nitzschia stagnorum

Fig. 13. Navicula oblonga

Fig. 14. Surirella ovata var. crumena

Fig. 15. Diploneis smithii var. laevis

Plate III

Fig. 16. Aphanizomenon flos-aquae f. klebahnii

Fig. 17. Aphanizomenon issatschenkoi

Fig. 18. Anabaenopsis raciborskii

Fig. 19. Lyngbya limnetica

Fig. 20. Nitzschia acicularis

Fig. 21. Merismopedia minima

Fig. 22. Pediatrum tetras var. tetraodon f. globosum

Fig. 23. Scenedesmus balatonicus

Fig. 24. Pediatrum tetras

Plate IV

Fig. 25. Pediatrum duplex

Fig. 26. Asterionella formosa

Fig. 27. Pediatrum simplex

Fig. 28. Pediatrum boryanum

Fig. 29. Navicula gastrum var. signata

Fig. 30. Pandorina morum

Scales in the order of Plates I—IV: 10 μ m.

Plate I



Plate II

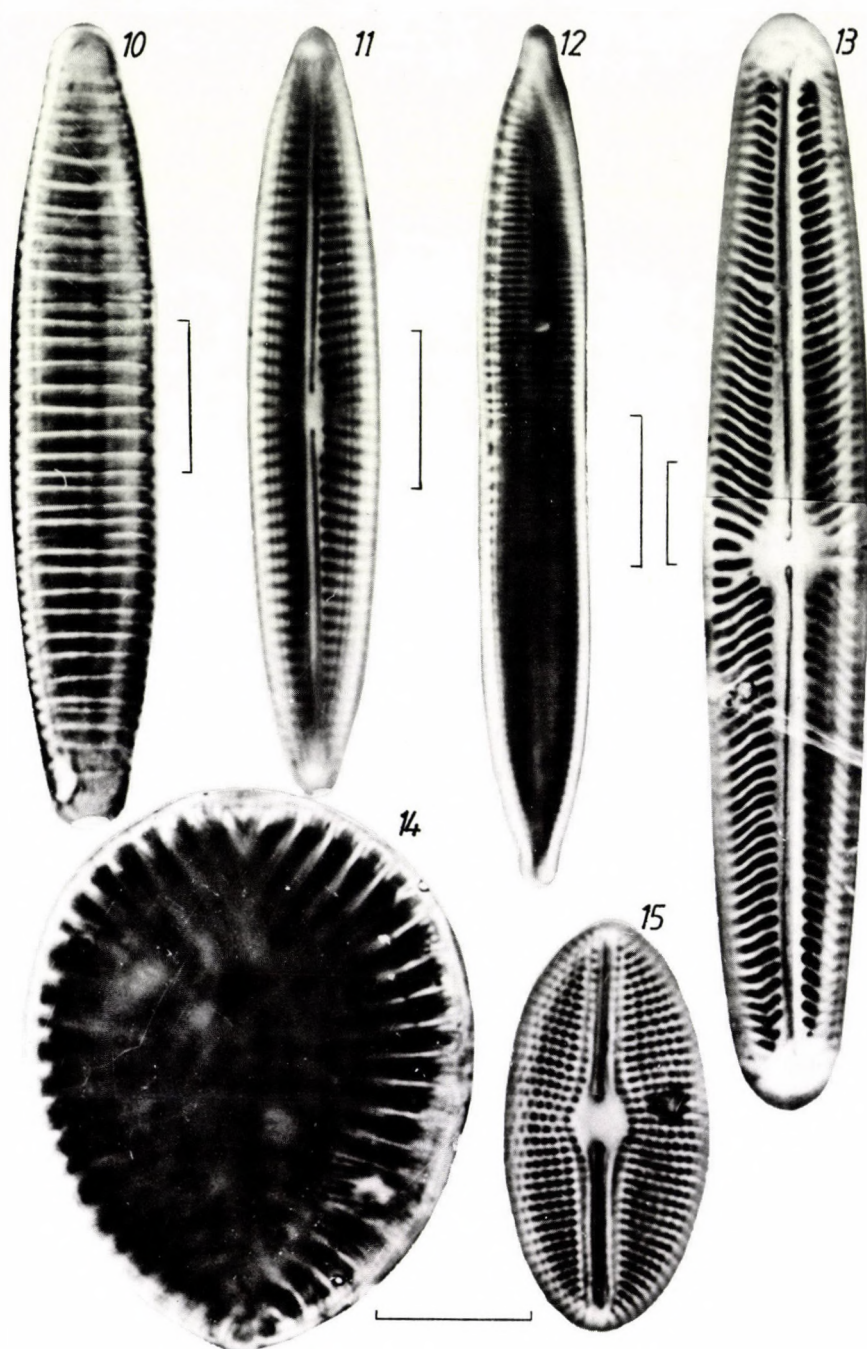


Plate III

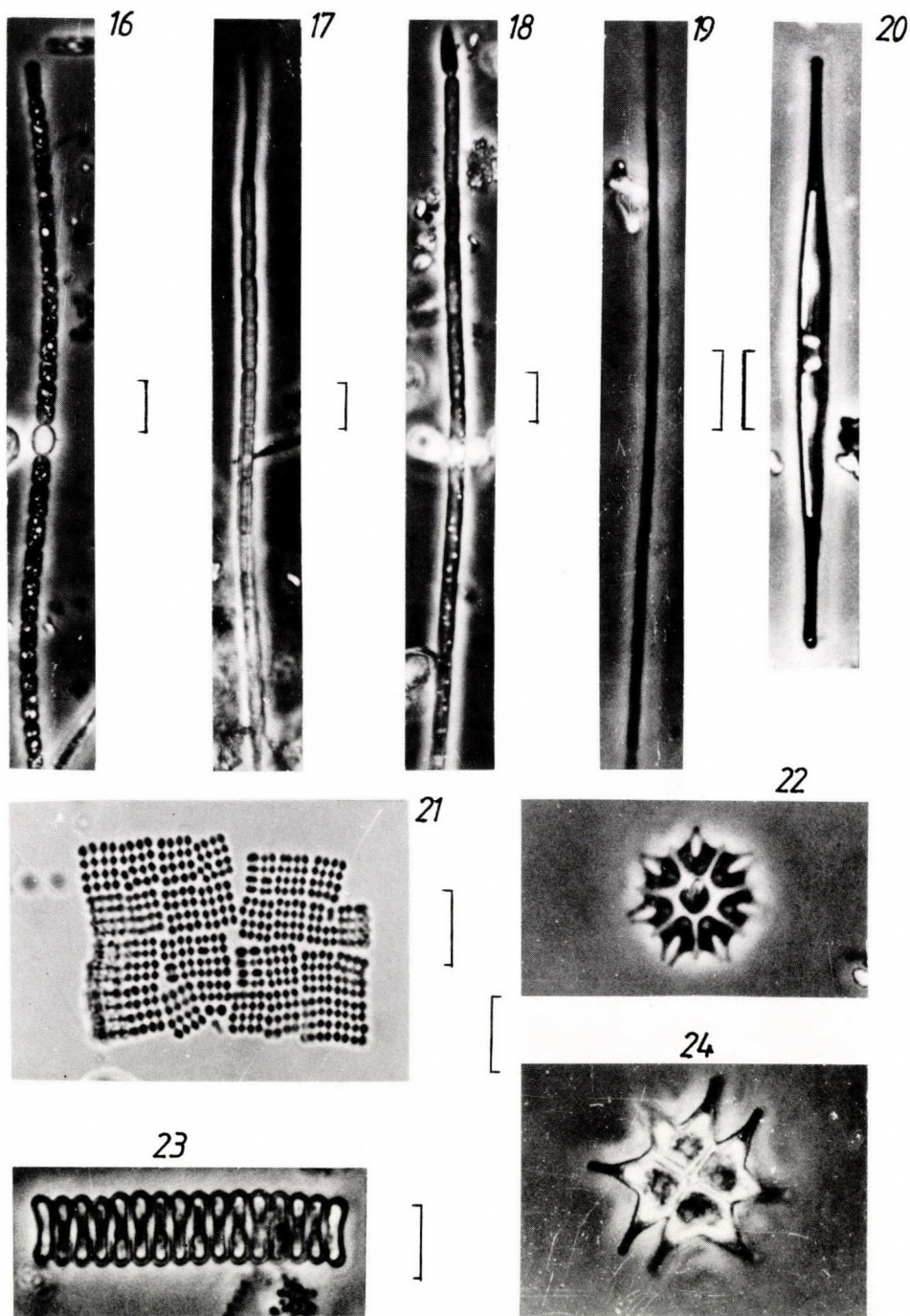
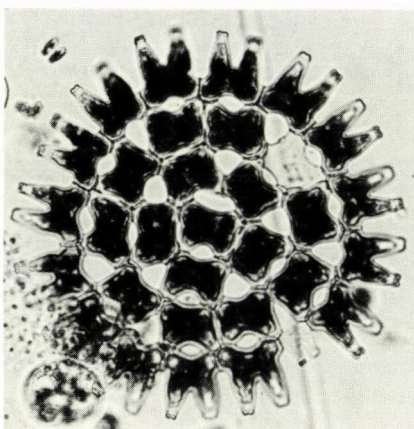
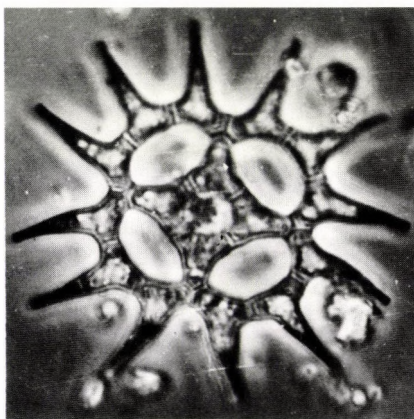
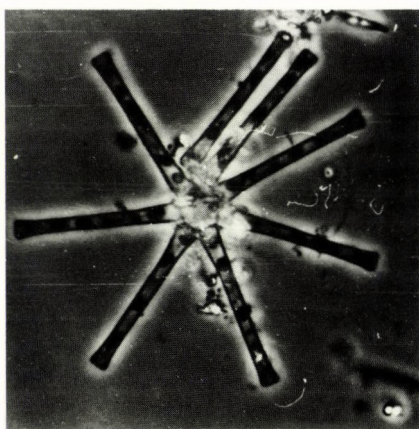


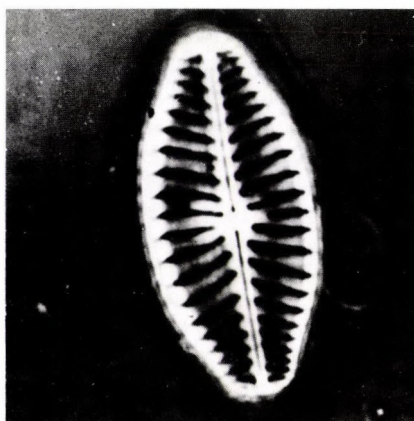
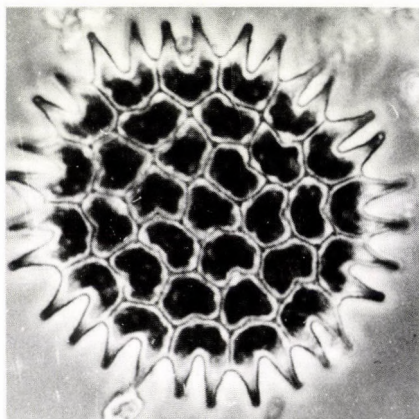
Plate IV



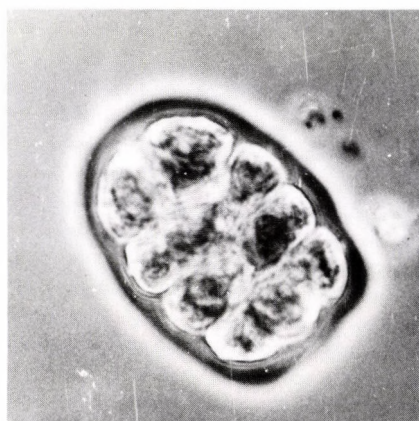
25 26



27 28



2930



IS THERE VEGETATION CONTINUUM IN MANGROVE SWAMPS?*

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Mangrove vegetation in southeastern Nigeria was sampled in eighty 100 m² quadrats regularly spaced along transects established from the shores inland. Vegetation measurements included frequency, density and coverage from which the importance values of species were computed. Species were assigned anthropogenetic adaptation numbers based on their relative importance and the continuum indices for all species in the quadrats were computed. PEARSON's correlation coefficients were also computed for all possible pairs of quadrats along the transects. A direct gradient ordination of species importance values on one composite transect revealed the existence of floristic gradations rather than discrete zonation of species from the shores inland. An ordination of PEARSON's r-values between adjoining composite quadrats indicated the existence of unstable conditions which accounted for the occurrence of overlap in species population modes. An indirect gradient ordination, based on the continuum index verified the existence of vegetation continuum in several understorey dominant mangroves. The dynamic equilibrium concept was proposed as an appropriate concept for the analysis of mangrove ecosystems.

Keywords: continuum index, dynamic equilibrium, floristic gradation, gradient analysis, mangrove vegetation, ordination, zonation

Introduction

Mangrove swamp forests are complex ecosystems that occur along intertidal accretive shores in the tropics (WALSH 1974). The swamps are dominated by specialized estuarine trees which generally occur in zones of species from the shores inland, and are often interpreted as seral stages in a hydrosere succession (CLEMENTS 1936; RICHARDS 1964; CHAPMAN 1976). The distinct belts of dominant mangrove species have also been described as separate plant communities (KASSAS and ZAHARAN 1967), which are related to salinity

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gradients and extents of tidal inundations (WEST 1956). As the mangroves appear to be discretely zoned at a broadly generalized level, few studies have analysed the commonly encountered aberrant patterns or quirks in species distribution (with the notable exceptions of THOM 1967; THOM *et al.* 1975 and RABINOWITZ 1978). The previous studies, being influenced by the classical Clementsian zonation and succession concepts resulted in numerous tidy zonation and succession schemes for different mangrove swamps and regions as reviewed by CHAPMAN (1976).

In the present study, the zonation concept is de-emphasized, based on the observation by THOM (1967) that the importance of physiographic change in mangrove habitats has not been fully considered as an ecological factor. Rather than infer a seral change, the distributional patterns in mangroves could be primarily a response to an ever-changing series of habitats which result from geomorphic changes associated with the development of the estuarine or deltaic plain. The implication is that since the swamp landscape is essentially unstable, the mangroves have utilized their halophytic adaptations to achieve a dynamic balance with the environment such that the species are always perpetuated along shorelines (as long as their environmental tolerance limits are not exceeded). Under this dynamic equilibrium notion, zonation *per se* cannot be said to occur in mangrove swamps since the dynamic conditions imply overlap both in vegetation and in the controlling factors of the environment. Consequently there may occur a continuous variation or vegetation continuum in the spatial distribution of species from the shorelines rather than sharp changes between vegetation zones.

Methods

To verify the existence or otherwise of a vegetation continuum in mangrove swamps, eighty 100 m² quadrats regularly spaced at 20-meter intervals along transects from the shorelines of three river estuaries in southeastern Nigeria were sampled, based on forest type differentiation. The forest types were modified after LUGO and SNEDAKER (1974) as (1) Distributary basin mangroves, (2) Point-bar mangroves, (3) Braided channel mangroves, (4) Inter-distributary basin mangroves, (5) Wooded levee mangroves, (6) Tributary creek mangroves, (7) Interriverine creek mangroves and (8) Beachridge strand mangroves. Forest type differentiation eliminated a bias for establishing transects on more accessible and commonly encountered habitats.

Vegetation measures for the overstorey (> 3 m) included crown cover by the crown-diameter method (MUELLER-DOMBOIS and ELLENBERG 1974), species density and frequency. Measures for the understorey (< 3 m) were obtained in 25 m² sub-quadrats, while samplings were in 1 m² subplots. Due to the presence of extensive props on most mangrove species, basal area was not used as a measure of dominance. The relative measures of frequency, density and coverage were summed to obtain the importance values for species.

In order to observe the peaks and species modalities from the shores inland, the importance values of species were ordinated on one composite vegetation transect, from which a direct environmental gradient relationship was inferred. The steepness of the gradient was further analysed by ordinating PEARSON's r -values beneath the vegetation transect (BESCHEL and WEBBER 1962). The PEARSON's correlation coefficients (r) were computed for all quadrat pairs in the swamps. This was based on the number of species in each quadrat which usually exceeded five (items); and although high correlations were encountered, these were exceptional rather than a general situation. The trends revealed by the mean r -values for composite quadrats were ordinated as direct gradient plots, with a transverse bar on each value indicating a probability of significance $P = 0.05$ from the t -test.

The existence of vegetation continuum was also investigated without direct reference to the spatial position of species on the landscape. In effect, this was an indirect gradient analysis approach based on computations of a continuum index for each quadrat (CURTIS and McINTOSH 1951). A primary step in the analysis was the computation of the importance value for each species in a quadrat and the leading dominant species in the quadrat identified i.e. the species with the highest importance value. Quadrats with the same species as leading dominants were grouped together and the average importance value for the species determined. By a subjective evaluation the species were arranged in an order such that the leading dominant occurring in the least number of quadrats and the leading dominant occurring in the largest number of stands were allocated the least and highest anthropogenetic "adaptation" numbers. The adaptation numbers were arbitrary numbers used in ordering species on a phytosociological scale of importance (KERSHAW 1973). The leading dominants occurring between the two established indices were accordingly given their own numbers based on the number of quadrats in which they were dominants. Therefore species which frequently occur together and may have similar environmental requirements have the same or similar adaptation numbers.

Table 1

Species importance values for overstorey mangroves averaged for six composite quadrats from the shorelines (1) to the inner swamps (6)

Species	Quadrats						Mean transect value
	1	2	3	4	5	6	
<u>Avicennia africana</u>	92.6*	84.7**	69.7*	46.9	29.3	42.9**	61.0
<u>Rhizophora mangle</u>	17.6	38.6	72.1**	64.3**	17.3	28.3	39.7
<u>Nypa fruticans</u>	54.3	59.7*	29.0	7.1	32.6	32.7	35.9
<u>Raphia vinifera</u>	0.0	2.5	23.6	61.6*	74.3**	37.9*	33.3
<u>Rhizophora racemosa</u>	105.1**	26.4	0.0	0.0	0.0	0.0	21.9
<u>Pandanus candelabrum</u>	7.1	6.0	17.6	34.6	12.1	0.0	12.9
<u>Rhizophora harrisonii</u>	2.1	25.7	3.6	13.4	17.1	10.0	12.0
<u>Triumfetta rhomboidea</u>	0.0	0.0	16.4	15.4	36.8*	2.9	11.9
<u>Phoenix reclinata</u>	0.0	1.4	9.6	5.0	4.6	8.7	4.9
<u>Drepanocarpus lunatus</u>	0.0	1.4	0.0	5.0	0.0	0.0	1.1
<u>Hibiscus tiliaceus</u>	0.0	0.0	0.0	0.0	4.3	0.0	0.7
<u>Conocarpus erectus</u>	0.0	3.1	0.0	0.0	0.0	0.0	0.5
Mean quadrat value	23.2	20.8	20.1	21.1	19.0	13.6	

* Co-dominant species

** Dominant species for each composite quadrat

The quadrat continuum index was derived by multiplying the importance value of each species in the quadrat by the adaptation number of the species which were then summed to obtain the total index for the quadrat. For the ordination, the importance values of species formed the vertical axis of a graphical plot while the quadrat continuum indices formed the horizontal axis. The representation was a scatter of points for each species through which lines of best fit were inserted to portray the ecological amplitudes of the species.

Results

Table 1 summarizes the results of vegetation analysis for the overstorey on one composite transect. The importance values (I.V.) of species were averaged for composite quadrats and arranged in spatial positions corresponding to the maximum number of quadrats and transect length of the widest forest type. Four species were observed to show similar ecological amplitudes across the transect. In order of importance the species were (1) Avicennia africana, (2) Rhizophora mangle, (3) Nypa fruticans and (4) Rhizophora harrisonii. The most important species at the channel margins were Rhizophora racemosa (I.V. 105.1) and A. africana (I.V. 92.6). Given a successional interpretation both species could be regarded as pioneer colonizers of mudflats, although SAVORY (1953) and KEAY (1953) regarded only R. racemosa as pioneer species. In the second quadrat A. africana (I.V. 84.7) and N. fruticans (I.V. 59.7) were the dominant species. But since N. fruticans is an introduced species (MERCER and HAMILTON 1984), its importance along the transect reflects the extent of human interference in the ecology of the mangrove swamps.

The middle of the transect was dominated by R. mangle (I.Vs. 72.1 and 64.3) in the third and fourth quadrats, respectively. R. mangle occurred in association with A. africana (I.V. 69.7) in the third quadrat and Raphia vinifera (I.V. 61.6) in the fourth quadrat. The inner swamps (quadrats 5 and 6) were dominated by R. vinifera (I.V. 74.3) and A. africana (I.V. 42.9) in association with Triumfetta rhomboidea (I.V. 36.8) and R. vinifera (I.V. 37.9), respectively. The species R. vinifera, T. rhomboidea and A. africana were the last landward major vegetation components of the vegetation transect. The highest mean importance value of 23.2 occurred at the shoreline and decreased to 13.6 in the last quadrat. Since these values showed a decrease inshorewards, a gradual floristic gradation was inferred during which the dominants replaced each other in importance along the transect. Clearly, there was no absolute discontinuity for the major species although certain minor associates e.g. Drepanocarpus lunatus, Hibiscus tiliaceus and Conocarpus erectus were restricted to narrow sections of the transect.

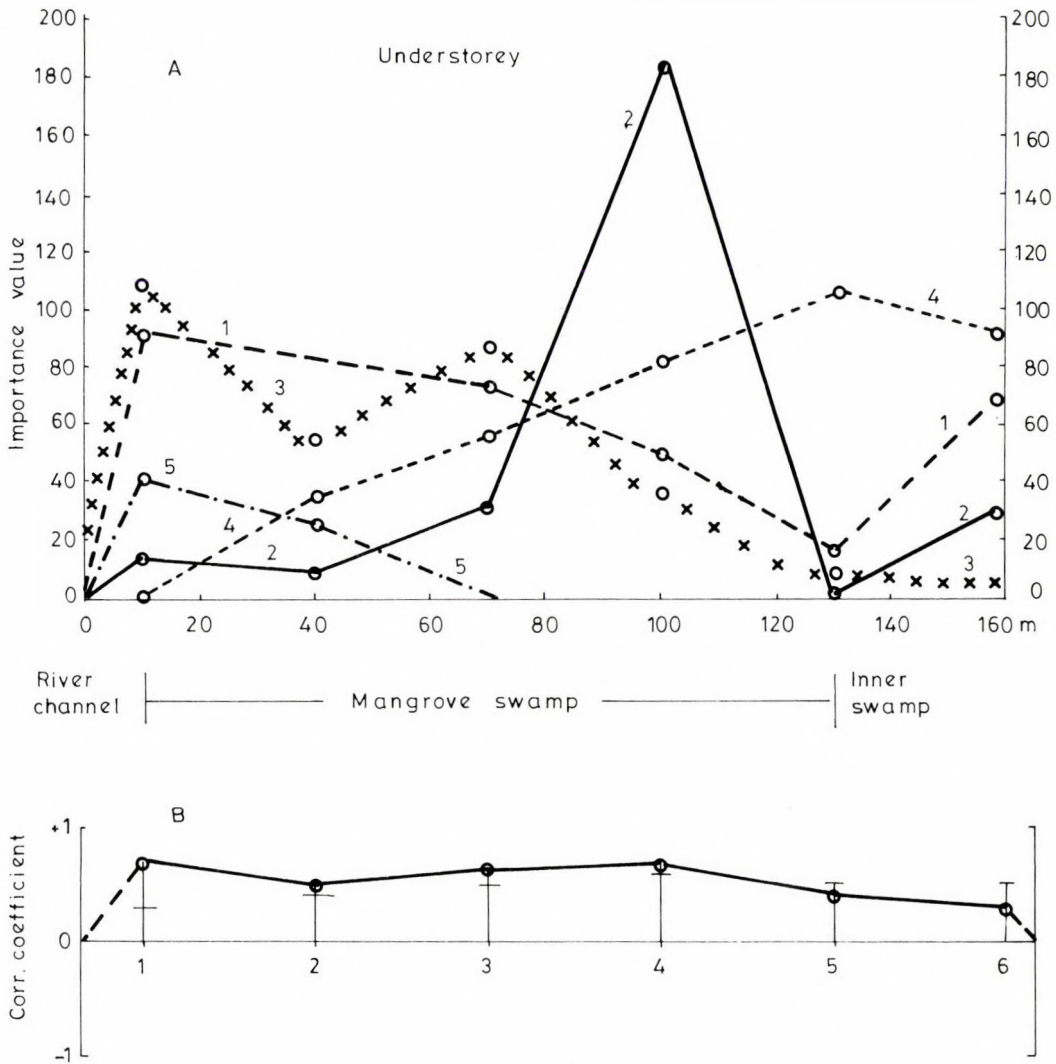


Fig. 1. Composite transect of mangrove swamp

A: Importance values for understory mangroves across six composite quadrats, (1) *Avicennia africana*, (2) *Rhizophora mangle*, (3) *Nypa fruticans*, (4) *Raphia vinifera*, (5) *Rhizophora racemosa*. B: correlation coefficients of the vegetation between adjoining quadrats. Probability at $P = 0.05$ level is indicated by -.

The ordination of species importance values and PEARSON's r -values for the understory vegetation is shown in Fig. 1. The trend of species modalities and peaks from the channels (upper diagram) showed that *A. africana*,

R. mangle, N. fruticans and R. vinifera had the widest ecological amplitudes. This was similar to the overstorey species modes in Table 1. Particularly, R. mangle appeared to depict a very steep gradient about the middle of the transect. However, the PEARSON's *r*-values (lower diagram) indicated that the species gradients were not steep; although the first four correlations were significant. There was a gradually sloping gradient from the first to the second quadrat, and a gently rising gradient to the highest positive correlation on the fourth quadrat. Then the gradient dipped into insignificant positive correlations on the fifth and sixth quadrats. Were the transect to be of greater length, it is possible that the correlations could have become negative, indicating the existence of a prominent ecotone at the mangrove/high forest margins. However, explanations for the insignificant correlations could be given in terms of (i) the presence of terrestrial deposits in the inner swamps with a consequent change in species composition, and (ii) the presence of silting ponds or permanently flooded areas that carry only groundlayer mangroves and samplings. Since the swamps experience diurnal flooding, the population modes reflect optimum adaptations to the environment. The sequence of dominance from the channels reflects largely the competitive abilities of the component species, e.g. species that dominate channel margins do so on the basis of suitably adapted rooting system, which is also a requirement for a wide amplitude across the unstable swamp landscape. Since the gradient (Fig. 1, lower diagram) dips gently towards the inner swamp, it is related to short-term changes in the swamp landscape (BESCHEL and WEBBER 1862), e.g. erosional and depositional tendencies that create zones of vigorous competition between species, such that the less vigorous species are replaced to relatively more stable (plateaux) areas along the gradient. As there is no positively correlated pair of composite quadrats (with similar *r*-values) that depict a uniformly stable (plateaux) condition, it implies that stable environmental condition which allows for the existence of sharp discontinuities in vegetation did not occur in the swamps.

Finally, a continuum index ordination was attempted for the understorey mangroves. This was because in each 100 m² quadrat, the importance values of understorey species could be obtained by summing the relative measures of frequency, density and coverage in four 25 m² subquadrats. The anthropogenetic "adaptation" numbers used for calculating the continuum indices are given in Table 2, while the average importance values for species used in the ordination are given in Table 3. The leading dominant occurring

Table 2

Anthropogenetic "adaptation" numbers for understorey mangroves and associates

Species	Adaptation numbers
<u>Laguncularia racemosa</u>	1
<u>Pandanus candelabrum</u>	1
<u>Triumfetta rhomboidea</u>	2
<u>Drepanocarpus lunatus</u>	2
<u>Raphia vinifera</u>	2
<u>Rhizophora harrisonii</u>	3
<u>Rh. mangle</u>	4
<u>Rh. racemosa</u>	4
<u>Avicennia africana</u>	5

in the least number of quadrats was Laguncularia racemosa while the leading dominant occurring in the largest number of quadrats was Avicennia africana. Both species represented the two extremes of an environmental gradient between which an interrelationship of environmental factors determined the importance of the other species.

Figure 2 shows the series of curves derived for five understorey mangrove species and associates, based on continuum index plots. The curves

Table 3

Average importance values for understorey mangroves and associates in 80 stands

Number of stands	Leading dominants	<u>A. africana</u>	<u>N. fruticans</u>	<u>R. racemosa</u>	<u>R. mangle</u>	<u>D. lunatus</u>	<u>R. vinifera</u>	<u>P. candelabrum</u>	<u>T. rhomboidea</u>	<u>L. racemosa</u>	<u>R. harrisonii</u>
29	<u>Avicennia africana</u>	200	17	53	25	7	-	2	-	-	11
4	<u>Nypa fruticans</u>	28	117	16	61	-	-	8	-	-	-
20	<u>Rhizophora racemosa</u>	35	34	214	6	-	2	8	-	-	4
14	<u>Rh. mangle</u>	23	22	-	179	5	11	24	7	-	4
1	<u>Drepanocarpus lunatus</u>	-	-	-	-	191	-	-	48	-	-
3	<u>Raphia vinifera</u>	-	-	-	-	-	161	17	15	-	-
5	<u>Pandanus candelabrum</u>	31	-	73	11	-	-	176	-	-	-
1	<u>Triumfetta rhomboidea</u>	-	-	-	29	-	-	131	143	-	-
1	<u>Laguncularia racemosa</u>	67	-	-	-	-	-	109	-	123	-
2	<u>Rhizophora harrisonii</u>	77	-	-	47	-	18	-	-	-	159

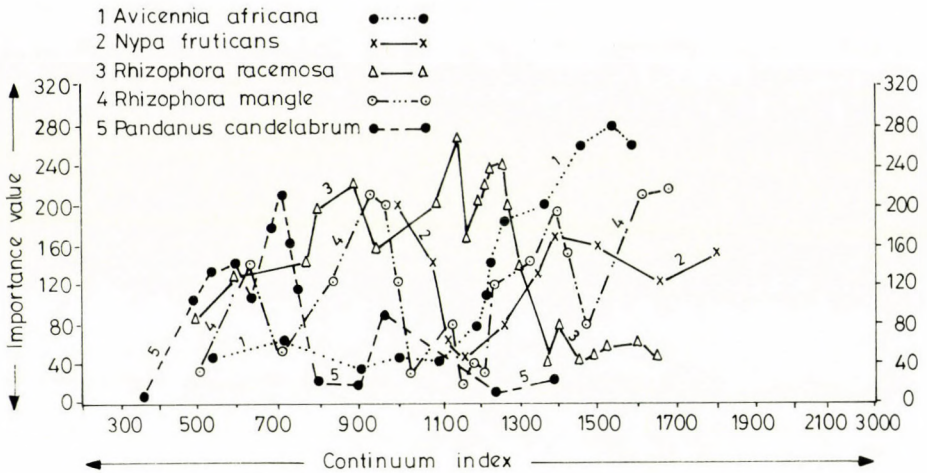


Fig. 2. Continuum index ordination for five understory dominants of the mangrove swamps

were drawn through scatter points of best fit. As pointed out by KERSHAW (1973), the fact that no groups of curves occur in zones of the continuum index imply that there is no discrete and recognizable associations within the vegetation community. In addition, a continuous overlap of many adjacent dominants (Fig. 2) was clearly demonstrated over a considerable range of the index in this study.

Conclusion

There is no doubt that, in detail, there exists a measure of vegetation continuum in mangrove swamps from the shores inland. This implies that environmental factors also display continuous gradation in consonance with the floristic pattern. Although vegetation zonation may be discerned at a broadly generalized level of investigation, it is apparent that the mangrove swamp being highly unstable does not allow for occurrences of the "tidy" species zones often discussed in the literature on mangroves. It appears that the dynamic equilibrium concept in which the vegetation is viewed as constantly adjusting to the unstable swamp landscape would be a more appropriate concept for the analysis of spatial patterns in mangrove vegetation.

This however is not a capitulation from the CLEMENTSian zonation viewpoint. Essentially, it is a proposal.

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REFERENCES

- Beschel, R., Webber, P. (1962): Gradient analysis in swamp forests. Nature 4824: 207-209.
- Chapman, V. (1976): Mangrove Vegetation. J. Cramer Publ. Co. Vaduz.
- Clements, F. (1936): Nature and structure of the climax. J. Ecol. 24: 252-284.
- Curtis, J., McIntosh, R. (1951): An upland forest continuum in the prairie-forest border region of Wisconsin. Ecology 32: 476-496.
- Kassas, K., Zahran, M. (1967): On the ecology of the Red Sea littoral salt marsh, Egypt. Ecol. Monogr. 37: 297-315.
- Keay, R. (1953): Rhizophora in West Africa. Kew Bull. 1: 121-127.
- Kershaw, K. (1973): Quantitative and Dynamic Plant Ecology. E. Arnold, London.
- Lugo, A., Snedaker, S. (1974): The ecology of mangroves. Ann. Rev. Ecol. Syst. 5: 39-64.
- Mercer, E., Hamilton, L. (1984): Mangrove ecosystems: some economic and natural benefits. Nature and Resources 20: 14-19.
- Mueller-Dombois, D., Ellenberg, H. (1974): Aims and Methods of Vegetation Ecology. J. Wiley, London.
- Rabinowitz, D. (1978): Early growth of mangrove seedlings in Panama and a hypothesis concerning the relationship of dispersal and zonation. J. Biogeo. 5: 113-133.
- Richards, P. (1964): The Tropical Rain Forest: An Ecological Study. C.U.P. Cambridge.
- Savory, H. (1953): A note on the ecology of Rhizophora in Nigeria. Kew Bull. 1: 127-128.
- Thom, B. (1967): Mangrove ecology and deltaic geomorphology, Tabasco, Mexico. J. Ecol. 55: 301-342.
- Thom, B., Wright, L., Coleman, J. (1975): Mangrove ecology and deltaic-estuarine geomorphology: Cambridge Gulf-Ord River, Western Australia. J. Ecol. 63: 203-232.
- Welsh, G. (1974): Mangroves: a review. In: Reimold, R., Queen, H. (eds): Ecology of Halophytes. Acad. Press, N. Y. 51-174.
- West, R. (1956): Mangrove swamps of the Pacific coast of Columbia. A.A.A.G. 46: 98-121.

ONTOGENETIC CHARACTERISTICS IN FLOWERS OF SOME PLUM CULTIVARS

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The author studied the flowers of plum cultivars budded on myrobalan (Prunus cerasifera) seedling between 1968 and 1989; the morphogenetic characteristics of the 13-34 years old trees were analysed with ontogenetic aspects taken into consideration. From the beginning of flower induction in June, the effect of year (mean temperature, monthly and annual amount of precipitation) produced many significant correlations, so the June-, July-, September- and February-mean temperatures as well as the July amount of precipitation were in significant correlation with the most important morphogenetic characters.

In the course of the 22 years the size of the petal changed the least, but the frequency of apistilled, polycarpous and pseudostaminate flowers showed intensive year effect. Differentiation in centripetal direction could be proved on the basis of defective flowers, since the destruction of pistil primordia brought about — on a several cultivar average — approximately a two-stamen surplus, irrespective of whether the number of androecia was two or three in the cultivars concerned.

The three life cycles (1968-1974, 1975-1980, 1981-1989) that could be continuously examined called attention to the fact that apart from the consequences of virus infection changes in the flower structure may be due to the age of life as well, as indeed, the morphogenetic and fertility conditions of the flowers of plum trees did change.

Further investigations are still necessary in the case of Prunoideae species, but the results obtained so far repeatedly call attention to the fact that it is not reasonable to establish a plum orchard consisting of a single (self-fertile) cultivar, particularly when we consider the economic losses of the period of decreasing yields.

Introduction

Apart from some exceptional cases in studies longer than ten years it is hardly usual to deal with the flower biology and -phenology of cultivars. Beyond the difficult nature of the work, disturbing factors due to the changing phytotechnical methods also play a role in this, and naturally the age of trees also changes in the meantime (STAUB 1982; HEDRICK et al. 1911; KEÖPECZY-NAGY 1943).

Shorter-term observations covering a large number of cultivars are, on the other hand, more frequent (DAHL 1935; RÖDER 1940; TÓTH 1957, 1975;

DERMINE and LIARD 1957, 1978; BRÓZIK 1960; SURÁNYI 1980a, b, 1985, 1986; TÓTH and SURÁNYI 1980). In fact, in most works descriptions of cultivars with morphological accuracy are found (MAS 1871—1875; BAILEY 1898, 1899; HEDRICK *et al.* 1991; DOMIN 1944; GOURLEY and HOWLETT 1941; TAYLOR 1949; KRJUKOV 1950; BROOKS and OLMO 1952; DERMINE and LIARD 1957, 1978; MORRISON 1964; BORDEIANU *et al.* 1963—1969).

The Hungarian plum descriptions (LIPPAI 1664—1667; BEREZKI 1877—1887; RUDINAI MOLNÁR and ANGYAL 1900; ANGYAL 1926; HERSZÉNYI 1934; RAPAICS 1935; TÓTH 1957; BRÓZIK 1960; TOMCSÁNYI 1960, 1980) contain highly valuable phenological observations too, built on the best Hungarian works (STAUB 1882; HEGYFÖKY 1926; KEÖPECZY-NAGY 1943), though in the subjects worked up we may find the major results of phenology recently pushed into the background in the international literature (SCHNELLE 1955; WILLING 1960; GARDNER *et al.* 1952; OVERCASH 1962; BORDEIANU *et al.* 1965; VONDRAČEK 1975).

In connection with the cultivars of the plum cultivar collection established in Cegléd in 1954/55 we have studied many a question and published the results; we encountered the problems of year effect first of all in our flower biology investigations (SURÁNYI 1978a, b, 1985), that we took into consideration in the phenological evaluations (SURÁNYI 1980a, b, 1986) and in the prognosis of the chemical fruit thinning effect (SURÁNYI 1990b), too.

Material and Method

In 1954/55 a large collection of plum cultivars was planted at Cegléd with trees budded on myrobalan seedlings in three replications. The major phenological characteristics of the trees were regularly recorded from the first blossoming, and in the meantime investigations of fertility biology and flower morphology were also carried on.

In the present study characteristics of flower morphology were examined for 12 plum cultivars from 1968 (the 13th year) to 1989 (the 34th year). In collecting the data and the flowers, respectively, we made use of experiences by GARDNER *et al.* (1952), KOBEL (1954) and TÓTH (1957), while in the evaluations works of SVÁB (1981), SCHUMACHER (1975) and TOMCSÁNYI (1980) were taken into consideration. The temperature and precipitation data were collected at the meteorological station of Cegléd.

Flowers at the beginning of pollen shedding were collected from the flowering spurs (SURÁNYI 1978a, 1985), only the most important morphogenetic characters of them were recorded during the 22 years. The length of peduncle and length and width of petals were measured in 30 flowers, from which the mean values were obtained; the pistil length, stigma diameter and relative stamen number were determined for each flower separately. Abnormalities appearing in the 30 flowers were also analysed every year; the frequency of defective and double pistilled flowers as well as of foliaceous stamina was both varietal and year effect.

The climatic data were evaluated from the beginning of flower-bud formation in most plum cultivars to the next flower induction, and not according to calendar year. Besides, the meteorological data of June and July were examined in details.

Correlation calculations cleared up possible connections between various characters, namely, to what extent the varietal and year effects were able to influence the different morphogenetic features, and above all, how the latter were affected by the climatic factors.

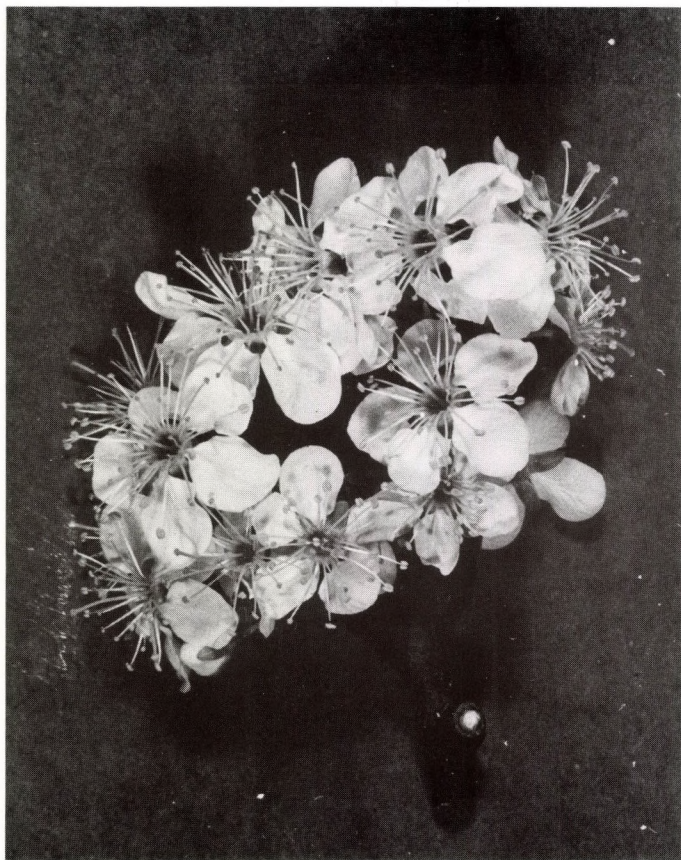


Fig. 1. Angouleme gage



Fig. 2. Besztercei plum IV.a 1/4



Fig. 3. Besztercei plum IV.b 25/2

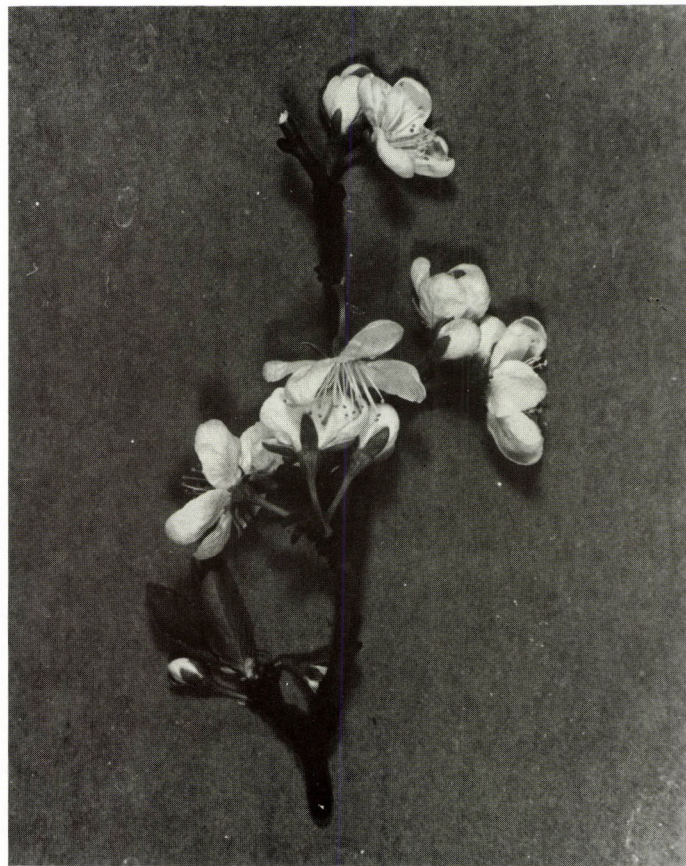


Fig. 4. Bosznia királynője (Queen of Bosnia)



Fig. 5. Böhli korai (Böhli early)

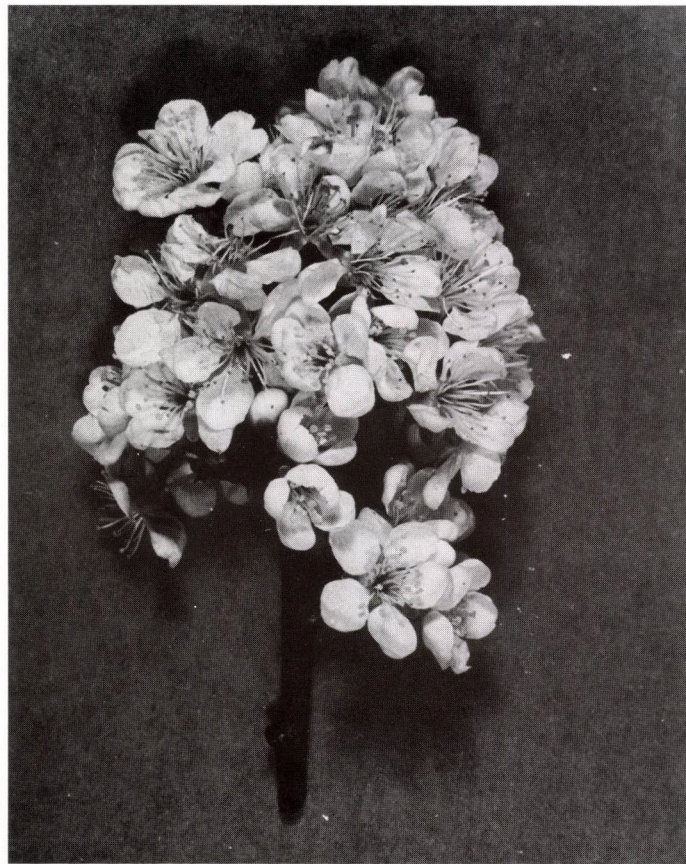


Fig. 6. Jodoigne gage



Fig. 7. Kék tojás (Blue egg)



Fig. 8. Olasz kék (Italian blue) = Nagy angol (Big English)



Fig. 9. Procureur



Fig. 10. Sermina = Vörös nektarin (Red nectarin)

The results of both the significance and the regression analysis are given in the paper. The 22-year period was broken up to three phases: 1968–1974, 1975–1980 and 1981–1989; the periodization was based on changes in the flower structure.

The 12 cultivars included in the study were: Angoulême and Jodoigne (gages); Beregi datolya and Kék tojás (egg plums); Procureur, Sermina and Tours (round plums), further the IV.a 1/4 and IV.b 25/2 clone cultivars of Besztercei szilva, Bosznia királynője, Böhli korai and Nagy angol (= Olasz kék) (sweet prunes). The cultivars were chosen from the collection on the basis of the relatively good health condition of the trees, the absence of virus symptoms, as well as with the continuity of the examinations and the pomological value taken into consideration.

Results and Discussion

The decisive period of flower-bud differentiation in the plum cultivars is in June, namely, the flower-bud formation begins 3–5 weeks after the phase of shoot growth, between the sprouting of the vegetative buds and the formation of flower-buds a definite time passes in each cultivar ($r=+0.970$) (SURÁNYI 1980b).

That is why we thought it important to analyse thoroughly the climatological characteristics of the months during the 22 years; it turned out that only the July and August mean temperatures did not significantly differ in the succession of months. It is important that on many years average the largest amount of precipitation fell in June, then in July; dry were, on the other hand, the late autumn and the winter (Table 1).

Table 1
Climatological characterization of months

Month	Average temperature (°C)	Average precipitation (mm)
June	19.7	67.1
July	21.4	53.4
August	20.7	46.6
September	16.9	37.4
October	11.1	28.7
November	4.9	44.3
December	0.8	39.4
January	-1.3	30.0
February	1.6	29.3
March	6.1	30.3
April	11.3	37.7
May	16.8	52.3
SD 5%	1.03	24.01

Table 2

Climatological characterization of the years of the study

Year	Annual mean temperature (°C)	Annual amount of precipitation (mm)	July precipitation (mm)
1967/68	11.9	363.4	38.9
1968/69	10.4	413.5	30.0
1969/70	10.7	635.1	22.1
1970/71	11.2	502.9	42.7
1971/72	11.5	463.0	80.7
1972/73	11.1	566.6	130.1
1973/74	11.7	422.5	62.5
1974/75	11.5	673.1	55.4
1975/76	10.2	639.1	115.5
1976/77	11.1	585.9	49.5
1977/78	9.8	412.4	39.4
1978/79	9.9	423.6	55.2
1979/80	10.1	478.0	69.5
1980/81	10.2	503.8	44.4
1981/82	10.2	495.5	52.4
1982/83	12.3	493.8	119.4
1983/84	10.9	395.9	16.0
1984/85	8.9	468.7	12.0
1985/86	11.5	505.9	34.3
1986/87	9.8	476.6	22.1
1987/88	11.6	515.3	38.5
1988/89	11.2	487.3	43.2
SD 5%	1.39	—	—

The so-called vegetation years reckoned from the beginning of flower-bud differentiation to the end of July next year show much more remarkable differences than what would be obtained with calendar years taken for basis (the average difference in scatter was 34.6%). The years 1977/78, 1978/79, 1986/87 and still more so 1984—85 excelled in coolness; in 1982/83 and 1967/68, on the other hand, the temperature mean of about 12 °C proved to be very high (Table 2).

Flower organization is influenced by the July amount of precipitation; in this extremely wide fluctuation was observed in the successive years of vegetation, but between the total amount of precipitation in the vegetation year and the July amount of it there was a slight positive correlation ($r=+0.329$). Beyond all that, in 1973, 1976 and 1983 the amount of rainfall at Cegléd was more than 100 mm. The fluctuation of the July value was 64.6%, while that of the vegetation years only 16.4%.

The most important characters are summed up in Table 3; the least change during the 22 years was shown by the mean value of the petal length;

Table 3

Flowers of twelve plum cultivars in the 22-year examination series (1968—1989)

Cultivars	Peduncle length	Petal size	Pistil length	Stamen number	Stigma diameter	Relative stamen number (n/mm)	Pollen germination	Apistilia	Polycarpy	Staminody
	(mm)	(mm)	(mm)	(n)	(mm)		(%)	(%)	(%)	(%)
Angouleme-i ringló	12.4	10.9	13.8	26.8	1196	1.95	50.6	4.0	0.3	0.1
Beregi datolya	12.6	10.3	13.4	19.1	807	1.43	54.7	2.4	0	12.7
Besztercei IV.a 1/4	13.2	10.2	14.1	19.8	1132	1.42	52.1	0	1.2	1.1
Besztercei IV.b 25/2	13.5	10.4	14.4	20.0	1289	1.38	41.8	0	0	11.5
Bosznia királynője	10.4	10.8	10.9	21.3	1068	1.94	57.1	0	0.2	0.9
Bühli korai	10.2	10.6	11.3	20.0	894	1.77	49.7	4.7	0	37.1
Jodoigue	9.9	8.9	10.6	27.4	979	2.62	64.3	1.1	9.8	1.7
Kék tojás	13.2	10.3	13.4	25.3	1165	1.89	38.9	1.9	0	1.5
Nagy angol (Olasz kék)	12.8	9.3	14.0	26.7	1113	1.87	56.0	1.0	0	3.6
Procureur	10.8	9.2	11.3	27.5	1170	2.46	56.0	9.2	0	0
Sermina (Vörös nektarin)	10.0	10.0	10.8	23.0	1089	2.14	39.8	0	0.6	2.9
Toursi nagy	9.5	9.8	10.6	22.7	982	2.17	49.9	2.5	0.1	0
CV, %	13.2	6.4	12.7	14.1	12.7	20.5	15.0	130.5	273.4	174.4
F-value	41.05	12.85	86.22	409.85	210.19	92.81	18.54	5.97	0.87	8.78
SD 5%	0.66	0.50	0.47	0.45	26.18	0.11	4.93	4.18	1.40	5.83

for the other characters 12-20% variability was observed. The abnormalities of flower, on the other hand, greatly depended on the environmental factors, on the climatic factors that we, too, examined; this was especially true for polycarpy.

The cultivars showed remarkable differences; the Tours and the Jodoigne had very short peduncles, while the peduncles of the two Besztercei clones and of the Kék tojás were conspicuously long. The petal measurements for the Jodoigne-, Procureur- and Nagy angol flowers are significantly smaller than those of the other cultivars, the Angouleme gage differentiates particularly large petals. The IV.b 25/2 and IV.a 1/4 clones of Besztercei szilva, and the Angouleme produced very long pistils, the Jodoigne and Tours excelled with short pistils.

According to Table 3 the stamen number of the cultivars shows a 14.1% fluctuation; the stamens are set in two or three circles; Beregi datolya, the two Besztercei clones and Böhli korai were found to have 20 stamens, while the gages examined were characterized by about 27 stamens. Although Böhli korai and Besztercei szilva are closely related to one another, they considerably differ in stigma diameter. In consequence of differences in pistil length and stamen number, the relative stamen number fluctuated: 1.40-2.60 stamens fell to one mm length of pistil.

Procureur (9.2%), Böhli korai (4.7%) and Angouleme gage (4.0%) often produced flowers without pistil; in these flowers at the beginning of the pistil primordium formation the sexual changes appear in the androecium as well:

<u>cultivar</u>	<u>normal</u>	<u>defective</u>
Procureur	27.5	29.1
Böhli korai	20.0	22.7
Angouleme gage	26.8	28.0

From this it is clear that the extent of stamen formation depends on the competitively influencing gynoecium too. The stamen number of the two kinds of flower caused a 7.9% difference, which produced an additional 1.83 stamens per flower in favour of the defective flowers (Table 3). The flowers change extremely in the case of defoliation caused by drought at the end of summer. On 12 November 1976 the Besztercei clone C. 970 blossomed the second time, and in the short-pistilled flowers 28 stamens differentiated, though they were not more than 18-20 at the normal (spring) time of opening (SURÁnyi 1977).

According to RÉMY (1954), MORRISON (1964) and TÓTH and SURÁNYI (1980) the pistil development depends more than the stamen number on the effect of environment and year. We found at Cegléd that the disorders of pistil organization (abortion) are increased not only by the break of summer bud dormancy (due to defoliation), but also by early flowering taking place after leafing (i.e. in cultivars with pistils of relatively late organization), e.g. in the case of *Walesi herceg*, *Korai kedvenc*, *Vörös nektarin* or *Nancy gage*. Pistil necrosis in autumn or early in winter is much more frequent than the former disorders (WATANABE and YASUNOBE 1961). The polycarpy of *Kirke szilvája* and *Stanley* is a phenotypic feature, but in some places it can be observed every year; other authors too have had similar experiences (TÓTH 1957; SURÁNYI 1985).

Of course, in spite of the strong environmental effect the flower abnormalities — whether it is apistilia, or the multiplication of pistils or even the phyllody of stamina — may also be cultivar characters (SURÁNYI 1990a). However, the early necrosis of pistil always resulted in the multiplication of stamina, since the direction of differentiation is centripetal (SURÁNYI 1972, 1976a, b). Yet, it would be an error to deny the role of other factors, as the morphogenetic and physiological effect of lamellae in apricot- and plum cultivars can be equally proved (SURÁNYI 1989).

Changes in the morphogenetic characters in the present study can be divided in three phases which are related with the age of trees and with climatic factors, so with the data of Table 4 summed up. Table 5 contains more information concerning the relationship between climate and flower organization. The genotypic effects generally are stronger than the phenotypic influences in the flowers of plum cultivars; it is the character rather than the direction of organization that the climatic factors are able to change. Irreversible effect can only be observed as the result of flower abnormalities (Table 4).

The changes caused by the effects of cultivar, rootstock and environment in the structure of flower are difficult to recognize and prove if only because the "range of action" may even vary from time to time. For example, the *Besztercei plum C. 970* forms different flowers on different rootstocks, but the length of peduncle and the pollen germination show definite year dependence (SURÁNYI 1990b). Practically the same rootstock effect was observed by Polish authors (GRZYB and ZAGAJA 1975).

During the 22-year series of examination the average peduncle length of the flowers gradually increased, some degree of significance could even

Table 4

Changes in the morphogenetic characters during the years examined

Years	Peduncle length	Petal size	Pistil length	Stamen number	Stigma diameter	Relative stamen number (n/mm)	Pollen germination	Apistilia	Polycarpy	Staminody
	(mm)	(mm)	(mm)	(n)	(mm)		(%)	(%)	(%)	(%)
1968.	11.4	9.9	12.2	23.6	1082	1.98	45.6	1.3	0.7	3.7
1969.	11.2	10.0	12.5	23.4	1049	1.92	47.3	2.2	1.4	5.8
1970.	10.9	9.8	12.3	23.5	1069	1.99	48.9	1.7	0.5	8.5
1971.	11.5	10.3	12.3	23.6	1099	1.95	45.4	0.8	2.2	2.5
1972.	11.6	10.1	12.1	23.6	1093	1.98	49.2	3.7	0.6	4.1
1973.	11.5	10.1	12.2	23.5	1069	1.96	47.6	2.3	0.3	11.0
1974.	11.7	10.3	12.2	23.6	1052	1.96	49.8	0.7	0.2	4.4
1975.	10.9	9.5	12.6	23.1	1095	1.87	49.3	2.4	0.6	6.5
1976.	11.3	9.4	12.7	22.8	1099	1.83	55.4	1.4	1.4	9.6
1977.	11.6	9.9	12.8	23.0	1084	1.81	46.3	0.6	0.7	2.5
1978.	11.5	9.6	12.8	23.4	1080	1.89	45.8	8.1	1.6	14.6
1979.	12.0	9.8	12.5	23.0	1048	1.84	52.6	11.7	0	10.9
1980.	11.5	9.6	12.8	23.1	1066	1.87	52.6	0	1.0	4.2
1981.	11.3	10.1	12.3	23.5	1082	1.95	52.8	3.2	1.4	11.1
1982.	11.6	10.4	12.5	23.2	1083	1.88	53.1	0.4	0.5	7.5
1983.	11.9	10.5	12.5	23.2	1084	1.88	53.3	0.9	1.9	3.3
1984.	11.6	10.6	12.2	23.3	1078	1.96	55.7	0.7	1.4	5.1
1985.	11.3	10.5	12.3	23.6	1048	1.95	53.2	1.3	2.1	5.2
1986.	11.5	10.5	12.1	23.3	1065	1.97	51.8	2.8	0.6	4.2
1987.	12.1	10.3	12.4	23.3	1087	1.94	56.3	0.9	1.3	1.3
1988.	12.3	10.3	12.3	23.3	1048	1.94	55.5	1.0	0.6	3.4
1989.	11.7	10.4	12.1	23.5	1062	1.98	52.5	1.1	1.6	4.8
CV, %	2.9	3.5	1.9	1.0	1.6	2.8	6.9	121.0	61.2	56.8
F-value	1.12	2.04	0.92	1.02	1.81	2.91	2.17	3.27	1.89	1.49
SD 5%	0.90	0.68	1.97	0.61	35.46	0.15	6.68	3.06	30.56	7.90

Table 5

Morphological characteristics of flower in different phases of bearing age
(plantation: 1954/55)

Characteristics	Phases of life			F-value
	1968-74	1975-80	1981-89	
Peduncle length, mm	11.4x	11.5	11.7x	1.87
Petal median, mm	10.18c	9.6AC	10.4aB	37.24 ⁺⁺⁺
Pistil length, mm	12.3B	12.7AC	12.3B	21.52 ⁺⁺⁺
Stamen number, n	23.5B	23.1AC	23.4B	18.05 ⁺⁺⁺
Stigma diameter, mm	1074	1079	1071	0.36
Relative stamen number, n/mm	1.96B	1.85AC	1.94B	2.05
Pollen germination, %	47.7C	50.3AC	53.8A	12.69 ⁺⁺⁺
Apistilia, %	1.8	4.0c	1.4b	2.06
Polycarpy, %	0.8	0.9	1.3	1.10
Staminody, %	5.7	8.1x	5.1x	1.42
Annual mean temperature, °C	11.2x	10.4x	10.7	1.50
Annual precipitation, mm	481.0x	535.3xx	482.5x	1.09
June temperature mean, °C	20.4x	19.6	19.3x	1.51
July temperature mean, °C	22.3b	20.4a	21.4	2.46
July precipitation, mm	58.1	64.1x	42.5x	0.90

+++ and ABC p = 0.1%

abc p = 5% or 1%

x and xx p = 10%

be established. Different, i.e. fluctuating was the trend of the petal median; the petals were smallest between 1975 and 1980, opposed thus to the amount of precipitation in the vegetation years. The water supply, on the other hand, was markedly proved by the pistil length, and was also indicated — in a different way again — by the stamen number; the characteristics of the two reproductive organs, on the basis of the relative stamen number, were in the closest correlation with the change of petal.

A genetic effect is suggested by the pollen germination, though beside the age factors the manifestation of certain plant sanitation effects could not be excluded either. In rainier years more abnormalities can be observed in the flowers of the plum cultivars, but the changes can be divided in two: they mean, on the one hand, the strengthening of the female character (increased size and number of pistils), on the other hand the dominance of the male character (more stamina, pollen, nectar etc.).

Table 6
r-Values of the most important correlations

Correlation	Cultivars n = 12	Years n = 22
Peduncle length—Pistil length	+0.976 ^{xxx}	+0.100
Petal median—Relative stamen number	-0.637 ^x	-0.529 ^x
Pistil length—Stamen number	-0.148	-0.638 ^{xx}
Pistil length—Pollen germination	-0.264	-0.114
Pistil length—Stigma diameter	+0.424	+0.219
Pistil length—Apistilia	-0.210	+0.090
Pistil length—Polycarpy	-0.331	+0.111
Stamen number—Staminody	-0.513 [Ⓢ]	-0.694 ^{xx}
Apistilia—Polycarpy	-0.177	-0.241
Polycarpy—Staminody	-0.164	-0.152

Ⓢ p = 10%
 x p = 5%
 xx p = 1%
 xxx p = 0.1%

The vegetation year was warmest between 1968 and 1974, though between 1975 and 1980 it was only cooler by 0.8 °C. The differences are but numerically considerable, statistically they hardly can be proved. Significant was, on the other hand, the change in the annual amount of precipitation and in the July precipitation from period to period. The June and July mean temperature was a less marked factor, though in groups of cultivar other regularities can also be observed (Table 5).

The more or less striking correlations are summarized by figures and in Table 6. Positive and negative trends equally occur in the graphic representation, and out of them the correlations of petal median and relative stamen number, of peduncle length and pistil length as well as of stamen number and number of phyllode stamina can be determined (Table 6).

To sum up the most important meteorological effects, from the point of view of morphogenetic characters the June, July, September and February effects of the climate are the most decisive. Warm weather in June and a relative deficiency of precipitation in July are favourable for the gynoecium, namely, the pistil becomes longer and the defective flowers decrease in number (Fig. 11A--B). Warm weather in July, on the other hand, is able to influence the petal size, but a July poor in precipitation is mainly favourable when the average temperature is above 22 °C, that is in hot days

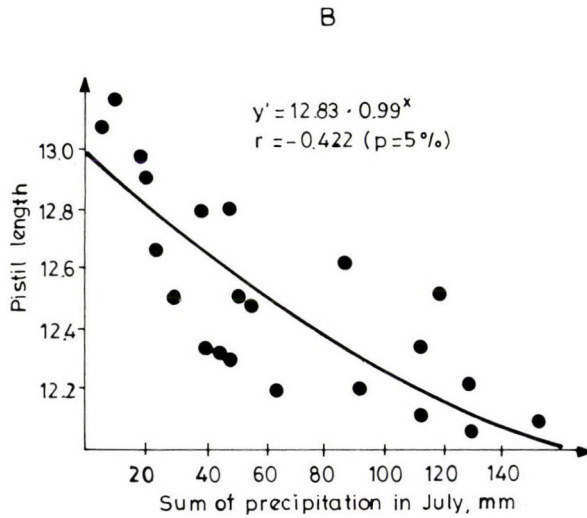
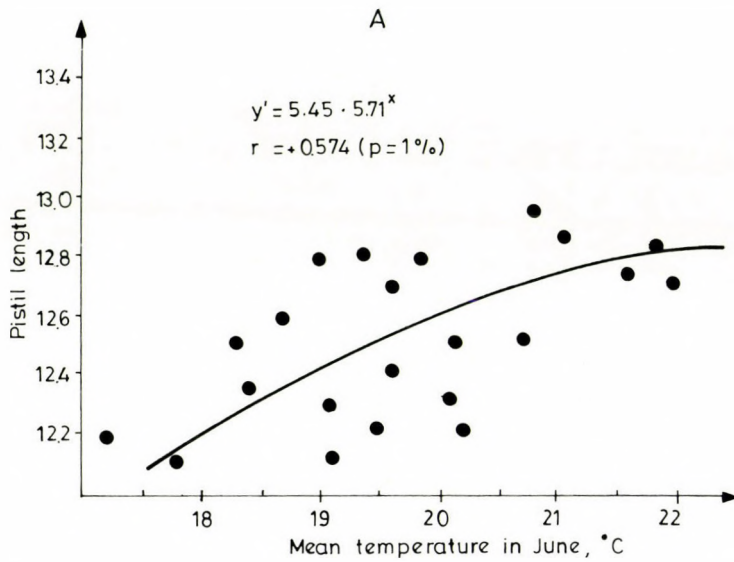


Fig. 11. Influence of June temperature mean and July amount of precipitation on pistil length (A, B)

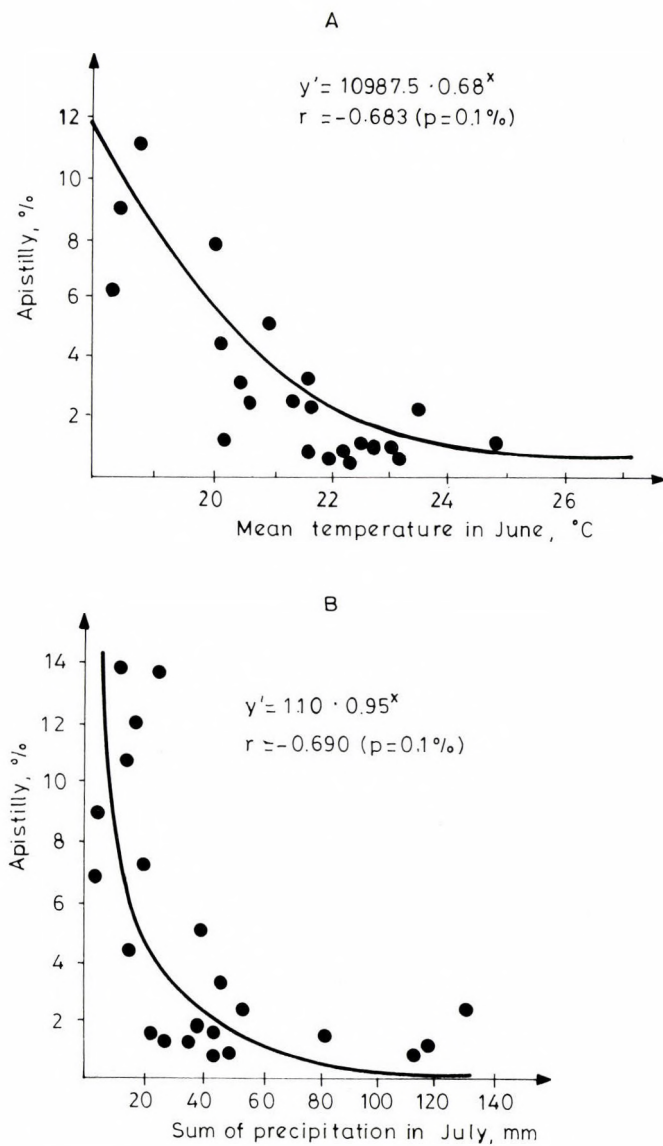


Fig. 12. Role of temperature mean and amount of precipitation in July (A, B) in the apistilia of plum cultivars

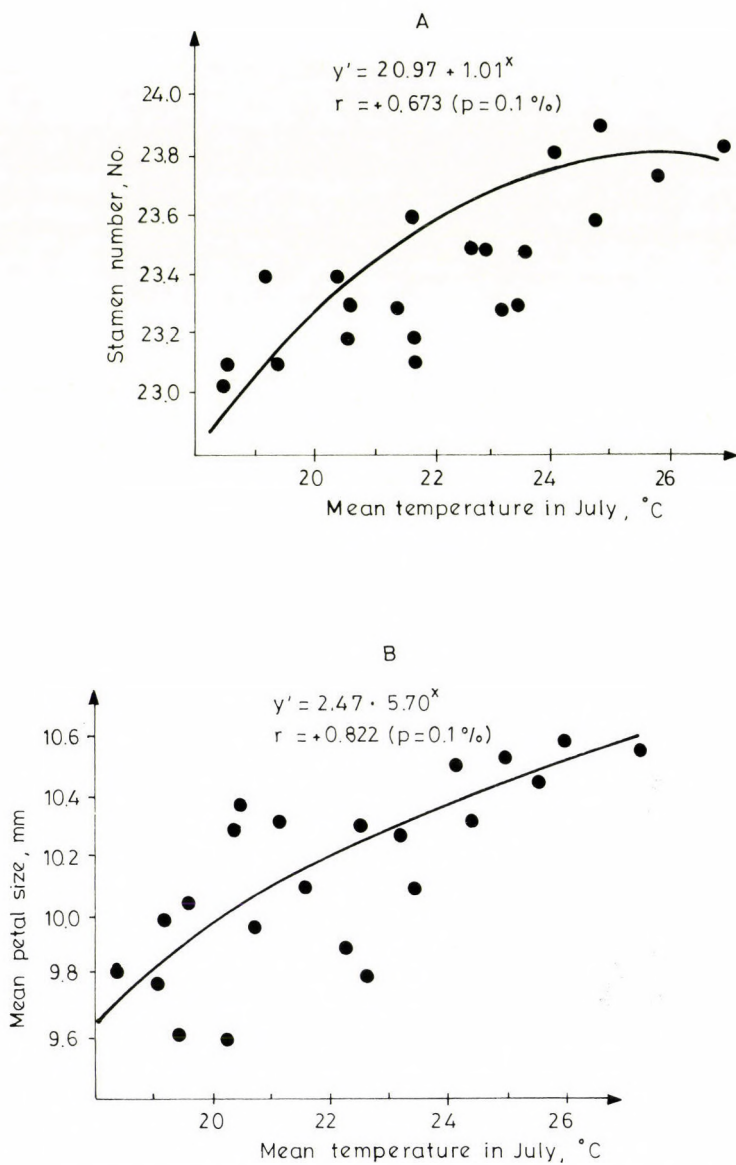


Fig. 13. Correlation of the July temperature mean with the actual stamen number and petal median (A, B)

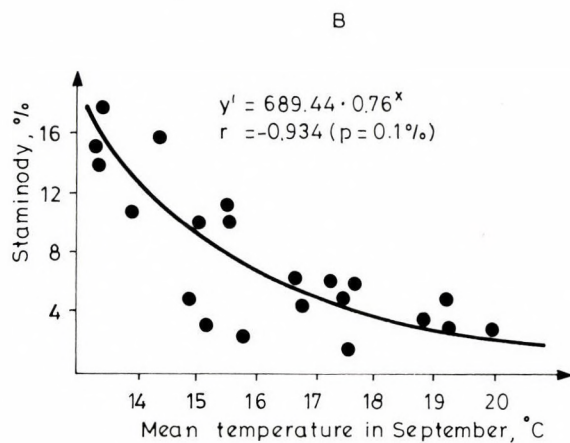
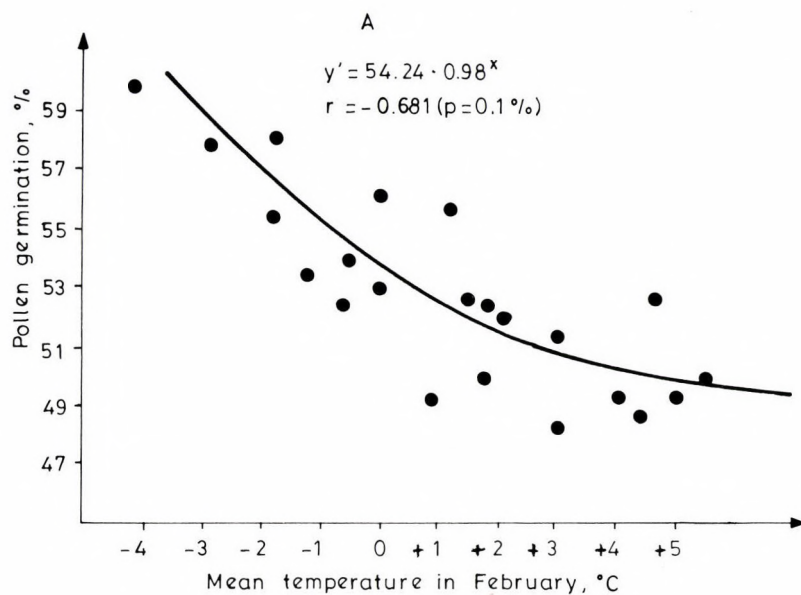


Fig. 14. Role of mean temperature in September (A) and February (B) in the organization of stamina and viability of pollen

(Fig. 12A--B). Similarly to the petal the stamina also require warm weather to grow in number (Fig. 13). In a cool, rainy September the anthers may become sterile in the form of staminodia and antherophylly in the course of stamen differentiation (Fig. 14). Some plum cultivars, e.g. Kék uri, Besztercei szilva, Violaszínű ringlő are particularly sensitive; i.e. there are genotypic teratomes as polycarpy of Stanley is a regularly recurring teratoma.

As regards ecological conditions, cultivars planted and rootstocks chosen it should be taken into consideration that optimum climate, soil conditions and site hardly exist in the case of plum. Yet, there are good cultivars, proper phytotechnical procedures which serve the very purpose of creating the conditions of balanced and large yield with biological means, supported by the mixed plantation of cultivars. This conception and practice is profitable not only in young plantations, but -- as in the present case -- in an old collection of cultivars too.

In spite of the present contingency of cultivars, the new results make it reasonable to carry out similar examinations in other species too, because even at the time of rapid changes of cultivar long-term experiments and decades of observation must not be regarded as unnecessary, since many problems only arise with older trees (cf. SURÁNYI 1985).

REFERENCES

- Angyal, D. (1926): Knowledge of fruit varieties. Athenaeum, Budapest.
- Bailey, L. H. (1898): Sketch of the evolution of our native fruits. MacMillan Co., New York.
- Bailey, L. H. (1899): Four report on Japanese plums. Bull. Cornell Univ. Agric. Exp. Sta. 198: 129--154.
- Bereczki, M. (1877--1887): Fruit growing sketches. I--IV. Réthy-Gyulai, Arad (in Hungarian).
- Bordeianu, T., Constantinescu, N., Stefan, N. (1963--1969): Pomologia Republicii Socialiste Romania I--VIII. Edit. Akad. R.S.R., Bucuresti.
- Brooks, R. M., Olmo, H. P. (1952): Register of new fruit and nut varieties 1920--1950. Univ. Calif. Press, Davis.
- Brózik, S. (1960): Plum -- Apricot (in Hungarian). Mezőgazdasági Kiadó, Budapest.
- Dahl, C. L. (1935): Morphological studies of plum flowers. Meded. perm. Komm. Fruktod. Förs. 38: 1--93.
- Dermine, E., Liard, O. (1957): Identification et description de variétés du prunier Européen. Encycl. Agron. Vétérin. Paris 1--322.
- Dermine, E., Liard, O. (1978): Identification et description de variétés du prunier Européen. II. partie. I.R.S.I.A. Gembloux.

- Domin, K. (1944): De origine prunorum diversi generis et fundamenta classificationis botanicae speciarum culturarum sectionis *Prunophora*. Bull. Inst. Trav. Prés. 45: 365—395.
- Gardner, V. R., Bradford, F. C., Hooker, H. D. Jr. (1952): The fundamentals of fruit production. McGraw-Hill, New York—Toronto—London.
- Gourley, J. H., Howlett, F. S. (1941): Modern fruit production. MacMillan Co., New York.
- Grzyb, Z. S., Zagaja, W. (1975): The influence of various rootstocks on the morphological changes in Italian prune flowers. Acta Hort. Hague 48: 7—11.
- Hedrick, U. P. et al. (1911): The plums of New York. Rep. N. Y. Sta. Agric. Exp. Sta. 3 (1): 1—616.
- Hegyföky, K. (1926): Data to fluctuation of the flowering time (in Hungarian). Matemat. Term. tud. Közl. 35: 115—163.
- Herszényi, L. (1934): Knowledge of fruit cultivars. Stephaneum, Budapest.
- Kobel, F. (1954): Lehrbuch des Obstbaues auf physiologischer Grundlage. Springer, Berlin—Göttingen—Heidelberg.
- Keöpeczy-Nagy, Z. (1943): Development stages of fruit-trees in Hungary (in Hungarian). Magy. Kir. Kert. Szőlő Főisk. Közlem. 9: 95—108.
- Krjukov, F. A. (1950): Plum varieties for the Leningrad province. Sad i Ogorod 6: 23—27.
- Lippai, J. (1664—1667): Garden of Pozsony. I—III. Cosmoverius M., Nagyszombat—Bécs.
- Mas, S. A. (1871—1875): Les Verger. Vols 6, 7 et 8. Libr de G. Marson, Paris.
- Morrison, J. W. (1964): The stamen number of some fruit species and varieties grown at Morden, Manitoba. Proc. Amer. Soc. Hort. Sci. 84: 123—130.
- Overcash, J. P. (1962): 50 plum varieties in Station Orchard: earliness studies. Miss. Fm. Res. 25: 7.
- Rapaics, R. (1935): Taxonomic place of the Hungarian plums. Bot. Közl. 34: 144—151.
- Rémy, P. (1954): Contribution à l'étude du pollen des arbres fruitiers à noyau, genre *Prunus*. Ann. Amél. Plant. 3: 351—388.
- Röder, K. (1940): Sortenkundliche Untersuchungen an *Prunus domestica*. Kühn-Archiv B. 54: 1—133.
- Rudinai Molnár, I.—Angyal, D. (1900): Hungarian pomology (in Hungarian). Athenaeum, Budapest.
- Schnelle, F. (1955): Pflanzenphänologie. Akad. Verlag, Berlin.
- Schumacher, R. (1975): Die Fruchtbarkeit der Obstgehölze. Ulmer, Stuttgart.
- Staub, M. (1882): The phenological map of Hungary. Matemat. Term. tud. Közl. 18: 1—28.
- Surányi, D. (1972): Teratological changes in *Prunus* varieties and their interpretation by the sex correlation between pistil and stamina. Bot. Közl. 59: 119—124.
- Surányi, D. (1976a): Differentiation of self-fertility and self-sterility in *Prunus* by stamen number/pistil length ratio.
- Surányi, D. (1976b): Evolutional aspects on the flower of *Angiospermae*. Biológia 24: 149—166.
- Surányi, D. (1977): Sexual studies in plum species. 1065th Meeting of HBS, 22nd Nov. 1977.
- Surányi, D. (1978a): A new method for determining self-fertility in plum varieties. Acta Agron. Hung. 27: 247—257.
- Surányi, D. (1978b): Morphogenetic characters and their relationships in gynoecium and androecium of some genera Prunoideae. Doctoral dissertation, Budapest.

- Surányi, D. (1980a): Comparative morphological and phenological study on plum varieties. Acta Agron. Hung. 29: 79–89.
- Surányi, D. (1980b): A study of some phenophases in plums. Acta Agron. Hung. 29: 265–282.
- Surányi, D. (1985): Flower structure of historical and cultivated plums, relationship between morphological characters and self-fertility. Candidate dissertation, Cegléd, Budapest.
- Surányi, D. (1986): Phenometrical characteristic of plums regarding the air temperature requirements of flowering and ripening. Acta Agron. Hung. 35: 63–78.
- Surányi D. (1988): The flower as character of cultivar in apricots and plums. Kertgazdaság 20: 15–27.
- Surányi, D. (1989): Number of leaf bud lamelles on apricot and flowers. Kertgazdaság 21: 72–82.
- Surányi, D. (1990a): Flower abnormalities as remarks of cultivar in plums. Kertgazdaság 22: 20–31.
- Surányi, D. (1990b): Climatic factors of the chemical thinning on stone fruit cultivars. Kertgazdaság 22: in press.
- Surányi, D., Tóth, E. (1977): Investigation of stamen-number in some plum varieties. Kertgazdaság 9: 41–51.
- Sváb, J. (1981): Biometrical methods in research work. Mezőgazdasági Kiadó, Budapest.
- Taylor, H. V. (1949): The plums of England. Crosby Lockwood and Son, London.
- Tomcsányi, P. (1960): Literature relations for fruit varieties. Agroinform, Budapest.
- Tomcsányi, P. (1980): Our fruit varieties. A practical pomology. Mezőgazdasági Kiadó (Agric. Press), Budapest.
- Tóth, E. (1957): Comparative biological and morphological studies on plum varieties. Kert. Kut. Int. Évk. 2: 11–129.
- Tóth, E. (1975): La sélection clonale de la variété Bestercei. Acta Hort. Hague 48: 111–120.
- Tóth, E., Surányi, D. (1980): Plum. Mezőgazdasági Kiadó, Budapest.
- Vondraček, J. (1975): The study of some phenophases in plums. Acta Hort. Hague 48: 23–34.
- Watanabe, T., Yasunobu, Y. (1961): Investigation on the development of flowers with defective pistils in peach and Japanese plum. Bull. Hort. Branch. Kanagawa Agric. Exp. Sta. 9: 41–44.
- Willing, H. (1960): Phänologische und chemische Untersuchungen zur Fruchtentwicklung bei Kirschen. Archiv f. Gartenbau 8: 561–594.

XYLOTOMICAL STUDY OF SOME VENEZUELAN TREE SPECIES (MIMOSACEAE I-IV)

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Fourteen legume tree species of Venezuela are xylotomically characterized (by K. BABOS) with the descriptions of external morphology and habitat conditions of each (contribution of L. J. C. CUMANA). The studied species are: Albizzia quachapele, A. lebbeck, Inga spuria, Acacia glomerosa, A. macracantha, A. tamarindifolia, Pithecellobium ligustrinum, P. tortum, Prosopis juliflora, Piptadenia flava, Mimosa arenosa, M. pigra, Leucaena latisiliqua and Enterolobium cyclocarpum.

Material and Methods

The blocks made of the wood of the four species were softened in a mixture of water and glycerin, in BRINZER's autoclave, at 1.5-2.0 atm, and then transversal, tangential and radial sections were prepared. The sections were stained with alcoholic solution of Toluidine-blue. The maceration of tissues was made with the SCHULZE-method (SÁRKÁNY and SZALAI 1964).

Length of fibres and vessel-elements, tangential and radial diameters of vessels, width and height of medullary rays and other characteristics were calculated from 50-100 measurements. Enlarged microphotographs were made of each section.

External morphology and distribution data are compiled by Prof. L. J. C. CUMANA.

External morphology

Albizzia quachapele (H.B.K.) Macbr.

Tree 15-20 m high, wide treetop, unarmed, foliage caducous. Leaves alternate, bipinnately compound, glandular, pubescent; the pinnae 3-7 pairs; the leaflets 5-8 pairs, 3-7 cm long, 2-2.5 cm wide, obovate or oblong, cuneate or acute basally, subemarginate apically. Inflorescence of tighten, globose, pedunculated capitulum-like spikes with numerous white-creamish flowers; the central flower very conspicuous and bigger than the others. Sepals 5, valvated, fused in a pubescent tube 12-14 mm long. Stamens 20-25, fused at the base, 40-49 mm long; anthers longitudinally dehiscent. Gynoecium 43-45 mm long, filiform, erect; ovary pubescent; stigma truncate.

Legume pubescent, chartaceous, 10-20 cm long and 1.5-2 cm wide. Autochthonous species known as "Samaniagua", "Acacia"; not very frequent, it grows on low soils of trophophyll and gallery forests.

Albizzia lebbeck (L.) Benth.

Tree 5-15 m high, wide treetop, pubescent toward young branches. Leaves alternate, bipinnately compound, glandular; the pinnae 2-4 pairs; the leaflets 3-9 pairs, 1.5-5 cm long, 15-20 mm wide, oblong or elliptic, rounded or submarginate apically, asymmetric basally. Inflorescence of tighten, globose, pedunculated, capitulum-like spikes with numerous fragrant yellowish or cream-greenish flowers; the central flower bigger than the others. Sepals 5, valvated, fused in a puberulent tube, 6-9 mm long. Stamens 30-32 fused in a tube; 2.5-3 cm long; anthers longitudinally dehiscent. Gynoeceum 2.5-2.8 mm long, glabrous. Legume oblong, chartaceous, glabrous, 15-20 cm long and 3-5 cm wide. Exotic species known as "Saman Margariteño", "Barba de Caballero", "Acacia". Frequently cultivated as an ornamental tree, sometimes it grows as an adventive in intervened zones. Easily adaptable to poor soils, very resistant to drought.

Inga spuria H. et B.

Tree 5-8 m high, generally wide treetop, tomentose in young branches. Leaves alternate, pinnately compound, glandular; rachis alate; the pinnae 4-7 pairs, 5-15 cm long, 2-6.5 cm wide, pubescent, oblong-lanceolate, acuminate or acute apically, obtuse basally. Inflorescence of rounded heads with numerous creamish flowers. Sepals 5, fused, valvated, 2-3 mm long. Petals 5, free, valvated, 4-5 mm long. Stamens 10, free, 9-10 mm long; anthers with scattered hairs, longitudinally dehiscent. Gynoeceum 8-9 mm long, filiform; stigma prominent; ovary pubescent toward the apex. Legume chartaceous, 10-17 cm long and 1.8-2 cm wide. Autochthonous species: not very frequent, it grows in trophophyllous forests as an adventive plant on uncultivated lands and in intervened zones of the hottest regions of the country.

Acacia glomerosa Benth.

Tree 5-10 m high, wide treetop, armed or unarmed, pubescent on the young branches. Leaves alternate, bipinnately compound, dark green above and pale green below, glandular, the pinnae 6-8 pairs; leaflets 12.25 pairs, 10-12 mm long, 3-4 mm wide, puberulent, linear-oblong, asymmetric basally,

acute apically, ciliate marginally. Inflorescence of rounded heads grouped in an attractive panicle with numerous white-creamish flowers. Sepals 5, valvate, puberulent, fused in a tube 1.5-2 mm long. Petals 5, valvate, fused in a pubescent tube 3-4 mm long. Stamens 75-85, somewhat united at the base, 5-6 mm long; anthers longitudinally dehiscent. Gynoeceum 5.5-6 mm long, stiped, erect; ovary pubescent. Legume compressed, chartaceous, glabrous, oblong, 10-20 cm long and 2-3 cm wide.

Autochthonous species known as "Caobano", "Mulato", "Tiamo"; typical in the hot regions of the country, frequent in thorn bushes, trophophyllous and dry forests of the Coastal Mountain Ranges, and plains.

Acacia macracantha H. et B.

Tree 5-7 m high, wide treetop, armed, foliage partially or totally caducous, branches conspicuously flexuous. Leaves alternate, bipinnately compound, glandular; pinnae 15-30 pairs, leaflets 20-40 pairs, 2.5-3.5 mm long and 1-1.5 wide, linear-oblong, oblique basally, obtuse apically, ciliate marginally. Inflorescence axillary of rounded heads with numerous flowers of a very intense yellow colour. Sepals 5, valvate, puberulent, fused in a tube 1-1.2 mm long. Petals 5, valvate, fused in a tube 2-3.5 mm long. Stamens 30-40, free, 3-4 mm long, arranged in two levels; anthers with a little prominent connective, longitudinally dehiscent. Gynoeceum 3-4 mm long, erect, shortly stiped; ovary glabrous. Legume subligneous, somewhat compressed, 6-10 cm long and 1 cm wide.

Autochthonous species known as "Yaque Hembra", "Cuji", "Yaque Hediondo", "Cuji Negro"; widely distributed in thorn bushland and dry and xerophytic forests of The Coastal Mountain Ranges, in plains and up to 1400 masl. Forage fruits.

Acacia tamarindifolia (L.) Willd.

Shrub 3-4 m high, armed, foliage caducous. Leaves alternate, bipinnately compound, glandular; the pinnae 4-6 pairs; leaflets 10-25 pairs, 10-12 mm long, 2.5-3 mm wide, glabrous, linear-oblong, oblique basally, acute apically. Inflorescence of rounded heads with numerous white-creamish flowers. Similar bracts and stipules, very conspicuous, cordiform, membranous, foliose, persistent, somewhat enveloping. Sepals 5, fused, valvate, 2-3 mm long. Petals 5, fused valvate, 4-6 mm long. Stamens 150-170, 12-14 mm long, somewhat united at the base; anthers longitudinally dehiscent. Gyno-

ecium 10-12 mm long, filiform, stiped with a conspicuously pilose ovary. Legume chartaceous, 10-15 cm long and 2-2.5 cm wide.

Autochthonous species known as "Chaguare" in some regions of the country: very common in xerophytic and trophophyllous forests, mainly in North Venezuela.

Mimosa arenosa (Willd.)Poir.

Shrub 2-4 m high, foliage caducous, armed or unarmed, pubescent toward young leaves. Leaves alternate, bipinnately compound, sensitive; pinnae 6-7 pairs; leaflets 10-16 pairs, 9-11 mm long, 2.5-3 mm wide, puberulent, oblong, acute apically, asymmetric basally. Inflorescence of attractive spikes with numerous white flowers. Sepals 4, 1-1.2 mm long, fused, valvate, ciliate apically. Petals 4, 2.5-3 mm long, fused, valvate. Stamens 8, 7.5-8 mm long, free, 4 longer than the others; anthers longitudinally dehiscent. Gynoeceum 6.5-7 mm long, asymmetric, glabrous, slightly stiped. Legume (craspedium); 7-10 segmented, chartaceous, glabrous, 4-7 cm long and 9-10 mm wide. Autochthonous species known as "Cuju", "Cujicillo", "Narualli"; widely distributed in the arid regions of North Venezuelan forming aggregates in trophophyllous and xerophytic forests.

Mimosa pigra L.

Shrub 2-3 m high, armed, hispid. Leaves alternate, bipinnately compound, sensitive, aculeate; pinnae 8-15 pairs, leaflets 30-40 pairs, 5-8 mm long, 1-2.5 mm wide, abaxial surface pubescent, conspicuously ciliate marginally, linear-oblong, asymmetric basally, subacute apically. Inflorescence of rounded heads with numerous pink flowers. Sepals laciniate, 1-2 mm long. Petals 4, pink, translucent, 2.5-3 mm long, fused, valvated. Stamens 8, 4 longer than the others, free, 7-8 mm long; anthers longitudinally dehiscent. Gynoeceum asymmetric, 5-6 mm long; ovary conspicuously pubescent. Legume (crasperium), 10-20 segmented, hispid, 6-10 cm long, 10-12 cm wide. Autochthonous species known as "Jalapatras", "Arestin"; very frequent in trophophyll and gallery forests, on low, flooded soils, in lagoons forming dense and impenetrable aggregates.

Enterolobium cyclocarpum (Jacq.) Griseb.

Tree 20-35 m high, wide treetop, unarmed, foliage caducous. Leaves, bipinnately compound, glandular; pinnae 4-12 pairs, leaflets 20-40 pairs, 3-13 mm long, 2.5-3 mm wide, oblong, oblique basally, acute apically. In-

florescence of tighten, globose, capitulum-like spikes with numerous cream-greenish flowers. Sepals 5, valvate, fused in a pubescent tube, 3-3.5 mm long. Petals 5, valvate, fused in a tube, 5-6.5 mm long. Stamens 60-62, 10-13 mm long, fused; anthers longitudinally dehiscent. Gynoeceum 10-12 mm long, filiform, glabrous, ovary conspicuously sulcate. Legume curved to circular, 8-10 cm diameter, subligneous, undulate marginally, slightly compressed between seeds. Autochthonous species known as "Caro", "Carocaró"; very common on low soils, trophophyllous forests, and galleries. Outstanding for its big size on river shores.

Leucaena latisiliqua (L.) Gillis.

Shrub 2-3 m high, unarmed. Leaves alternate, bipinnately compound, glandular; rachis and petiole pubescent; pinnae 7-8 pairs, leaflets 10-18 pairs, 8-10 mm long, 1.5-2 mm wide, oblong, asymmetric basally, acute apically, ciliate marginally. Inflorescence of rounded heads with numerous creamish flowers. Sepals 5, fused, valvate, 2-3 mm long. Petals 5, free, valvate, 4-5 mm long. Stamens 10, free, 9-10 mm long; anthers with scattered hairs, longitudinally dehiscent. Gynoeceum 8-9 mm long, filiform; stigma prominent; ovary pubescent toward the apex. Legume chartaceous, 10-17 cm long and 1.8-2 cm wide. Autochthonous species not very frequent, it grows on trophophyllous forests as adventitious plant, on uncultivated lands and intervened zones of the hottest regions of the country.

Pithecellobium ligustrinum (Jacq.) Klotzh.

Shrub or tree 3-12 m high, armed or unarmed. Leaves alternate, bipinnately compound, glandular; the pinnae one pair; leaflets one pair, 3-7 cm long, 2-3 cm wide, oblong-lanceolate, oblique basally, obtuse or rounded apically. Spikes with numerous white or white-creamish flowers. Sepals 5, valvated, fused in a tube, 1-2.5 mm long. Petals 5, valvated, pubescent, fused in a tube, 4-5 mm long. Stamens 32-37, 15-17 mm, fused in a tube; anthers longitudinally dehiscent. Gynoeceum 12-14 mm long, filiform; ovary pubescent. Legume subcylindric, the valves twisting after dehiscence. Autochthonous species known as "Bobo", "Orore"; very frequent in hot regions of the country, intergrating trophophyll and gallery forests, on low flood-plain and marshes. In general, on alluvial soils forming aggregates.

Pithecellobium tortum Mart.

Shrub or tree, 3-7 m high, foliage caducous, wide treetop, armed, exfoliating periderm in very conspicuous plates. Leaves alternate, bipinnately compound, glandular; rachis and petiole puberulent, oblong elliptic, asymmetric basally, subacute apically, ciliate marginally. Inflorescence of tighten, globose, capitulum like spikes with numerous white-creamish flowers. Sepals 5, valvated, fused in a somewhat zygomorphic tube, 1.5-2 mm long. Petals 5, valvate, conspicuously pilose toward the apex, fused in a tube, 4-4.5 mm long. Stamens 10-12, fused in a tube, 10-12 mm long; anthers longitudinally dehiscent. Gynoeceum 10-11 mm long, filiform, erect, glabrous, slightly asymmetric. Legume ligneous, straight or subcurved, compressed, glabrous 9-10 cm long and 1 cm wide. Autochthonous species known as "Cuji"; not very frequent, grows in xerophytic and trophophyll forests of hot regions of the country.

Prosopis juliflora D.C.

Tree 4-10 m high, armed, generally wide treetop, foliage partially caducous. Leaves alternate, bipinnately compound, glandular; pinnae 1-3 pairs, leaflets 10-15 pairs, 5-10 mm long, 1.5-3 mm wide, linear-oblong, asymmetric basally, subacute or obtuse apically, ciliate-margined. Spikes with numerous yellowish flowers. Sepals 5, valvated, fused in a tube, 1-1.5 mm long. Petals 5, valvate, free, lanate in the inner face, 2.5-3.0 mm long and 1.0-1.5 mm wide. Stamens 10, 3-4 mm long, free; anthers with caducous apical glandule, longitudinally dehiscent. Gynoeceum 4.5-5.0 mm long, erect; ovary pubescent. Legume subligneous, ceraceous, flattened linear or curved, 10-15 cm long, 6-16 mm wide. Autochthonous species known as "Yaque", "Cuji"; widely distributed around the country, from sea level to 1500 m above sea level, integrating different kind of forest such as desertic zones under very severe and hostile environmental conditions, xerophytic forests thorn bushes, trophophyllous forests and galleries. Easily adaptable to different types of soil, from poor dry soils where only few species can grow to humic, flooded and salty soils; also, on transitional zones as adventive in intervened and polluted zones. Forage fruits.

Piptadenia flava (Spreng.) Benth.

Shrub 2-4 m high, armed, foliage caducous. Leaves alternate, bipinnately compound; rachis and petiole puberulent; pinnae 8-12 pairs, leaflets 12-34 pairs, 3-8 mm long, 1-1.5 mm wide, linear-oblong, asymmetric basally,

acute or subacute apically, ciliate marginally. Spikes with numerous brown-reddish or yellow-reddish flowers. Sepals 5, fused, valvate, 1-2 mm long. Petals 5, fused, valvate, 3-4 mm long. Stamens 10, free, 5 longer, 4-5 mm long; anthers longitudinally dehiscent. Gynoecium 2-3 mm long, stiped, asymmetric, glabrous; style conspicuously curved. Legume chartaceous, 5-9 cm long and 10-12 cm wide. Autochthonous species known as "Cuji", "Cujicillo". Frequently integrating xerophytic forests; mainly distributed in arid and desert zones of North Venezuela.

Wood anatomy

Albizzia quachapele (H.B.K.) Macbr. (Table 1, Figs 1--3)

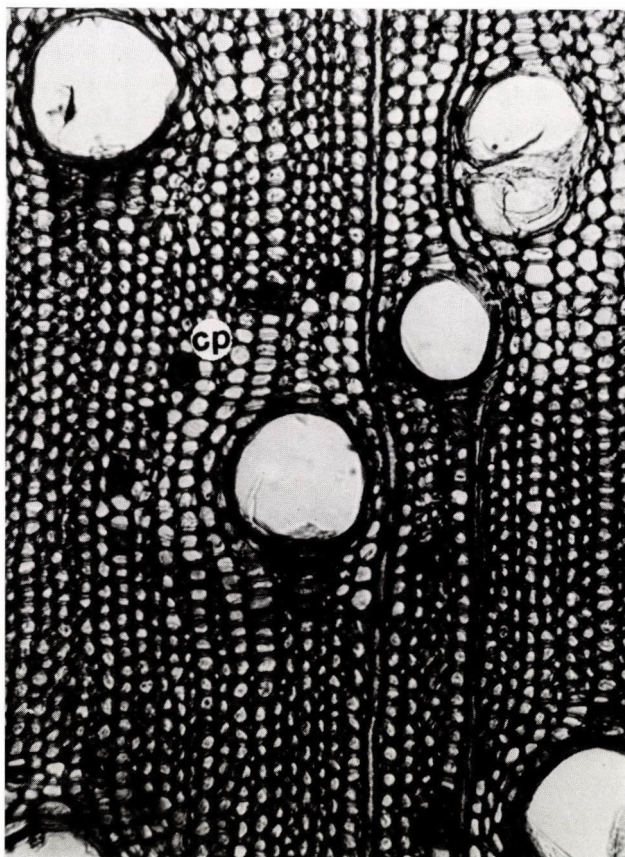


Fig. 1. Albizzia quachapele (H.B.K.) Macbr. Cross-section 120x. Vessels, medullary rays, fibres. Contact-vasicentric and aliform-confluent longitudinal parenchyma. In the longitudinal parenchyma cells crystals (crystal holder longitudinal parenchyma — cp)



Fig. 2. *Albizzia quachapele* (H.B.K.) Macbr. Tangential longitudinal section 120 x. One- or two-cell wide medullary rays, longitudinal parenchyma and fibres. Longitudinal parenchyma with calcium oxalate crystals (crystal holder longitudinal parenchyma = cp)

Wood diffuse-porous. The bulk of wood consists of thin-walled fibres of polygonal shape. The longitudinal parenchyma is contact-vasicentric and aliform-confluent. In the longitudinal parenchyma cells diamond-shaped calcium oxalate crystals. The medullary rays are narrow (Fig. 1).

The tracheae are oval, generally solitary, sometimes in pairs; in infrequent groups (of 3-5) tangentially flattened. Their number is 5-10.8-17/mm². The tangential diameter is 60.45-110.11-162.75 μm , the radial diameter 60.45-121.27-186.00 μm . The vessel members are 172.50-248.86-379.50 μm long, with alternately set medium size, elongated bordered pits in the walls. The perforation plate is simple.

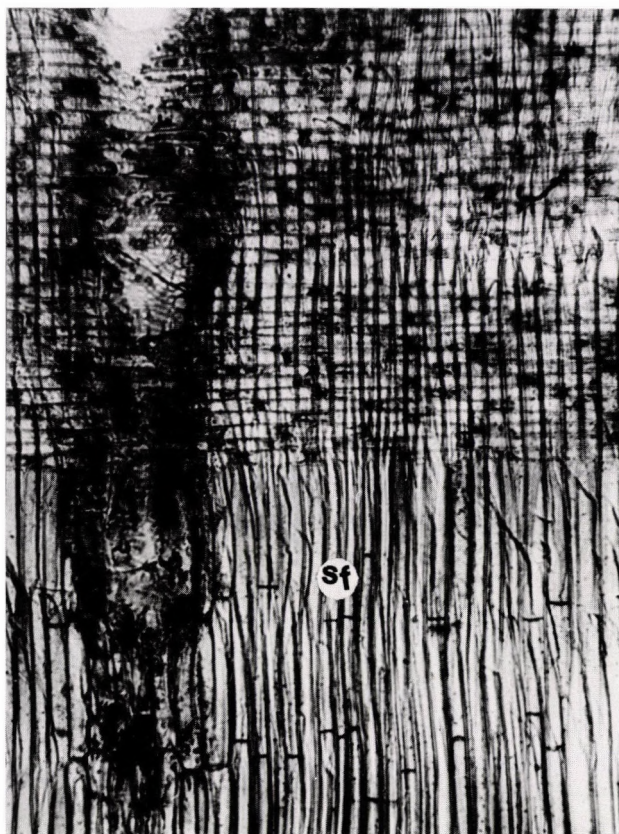


Fig. 3. *Albizzia quachapele* (H.B.K.) Macbr. Radial section 120 x. Homogeneous medullary rays, vessel and fibres. Septate fibre (sf)

The medullary rays are 1-2-3-cell wide with homogeneous structure; 69.00-214.36-575.00 μm in height and 11.50-19.78-34.50 μm in width (Figs 2, 3).

The fibres are arranged in radial rows, septate fibres are frequent. The diameter of fibre is 9.30-16.93-23.25 μm , the wall thickness 1.16-1.72-2.32 μm . The total length of fibres is 426.0-601.37-781.0 μm . The fibres end in smooth tips though sometimes they are serrate at one end.

The tangential diameter of longitudinal parenchyma cells is 11.50-19.78-34.50 μm . Their height is 69.00-101.84-167.40 μm . Often the cells contain mastic material. Crystal holder longitudinal parenchyma is not rare.

Table 1
Anatomical features of the species examined

Wood elements	Features	Albizia quachapele	Albizia lebbeck
Trachea	arrangement	diffused, solitary and duplicate, rarely in radial groups of 3-5 members	diffused, solitary and duplicate rarely in radial groups of 3 members
	shape	oval, shaped in groups in tangential direction flattened	oval, shaped in groups in tangential direction flattened
	tangential diam.	60.45-110.11-163.75 μm	55.8-113.46-172.05 μm
	radial diameter	60.45-121.27-186.00 μm	55.8-117.74-186.00 μm
	length of vessels	172.5-248.86-379.5 μm	138.0-222.41-322.0 μm
	number per mm^2	5.0-10.8-17.0	9.0-14.07-25.0
	wall thickness	3.48-6.07-9.30 μm	4.65-6.88-9.30 μm
	intervascular pitting	elongated bordered	elongated bordered
	perforate plate	simple	simple
	content	—	rarely mastic material
Medullary rays	width	narrow	narrow
	number of cells	1-2-3	1, rarely 2
	classification	homogeneous	homogeneous
	height	69.0-214.36-576.0 μm	34.5-116.38-218.5 μm
	width	11.5-19.78-34.5 μm	11.5-13.8-17.25 μm
	content	—	mastic material, crystal
Fibres	arrangement	radial rows	radial rows
	shape	polygonal	polygonal
	full thickness	9.3-16.93-23.25 μm	9.3-16.65-23.25 μm
	wall thickness	1.16-1.72-2.32 μm	1.16-2.44-4.65 μm
	full length	426.0-601.37-781.0 μm	497.0-831.41-1065.0 μm
	type of pitting	small, bordered	small, bordered
	other	septate fibre	septate fibre, crystal
Longitudinal parenchyma	arrangement	contact-vasicentric and aliform-confluent	contact-vasicentric and aliform-confluent
	diameter	11.5-19.78-34.5 μm	13.95-18.14-23.25 μm
	height	69.0-101.84-167.4 μm	55.8-82.03-139.5 μm
	number of cells	2-4	2-4
	content	mastic material	mastic material
	other	rarely septad crystal-holder longitudinal parenchyma	septad crystal-holder longitudinal parenchyma

Albizzia lebbeck (L.) Benth. (Table 1, Figs 4--6)

Diffuse-porous wood. The bulk of wood is made up of relatively thin-walled fibres of polygonal shape. The longitudinal parenchyma is contact-vasicentric and scanty aliform-confluent. On the borders of the growth zones 1-2-cell wide longitudinal parenchyma of terminal position is found. The medullary rays are narrow (Fig. 4).

The tracheae are oval, solitary or in pairs. Groups of 3 are very infrequent; here the trachea are tangentially flattened. The number of tracheae is 9-14.07-25/mm². The tangential diameter is 55.80-113.46-172.05 μ m, the radial diameter 55.80-117.74-186.00 μ m. The vessel members are 138.00-222.41-186.00 μ m long, with alternately set medium size, elongated, some-

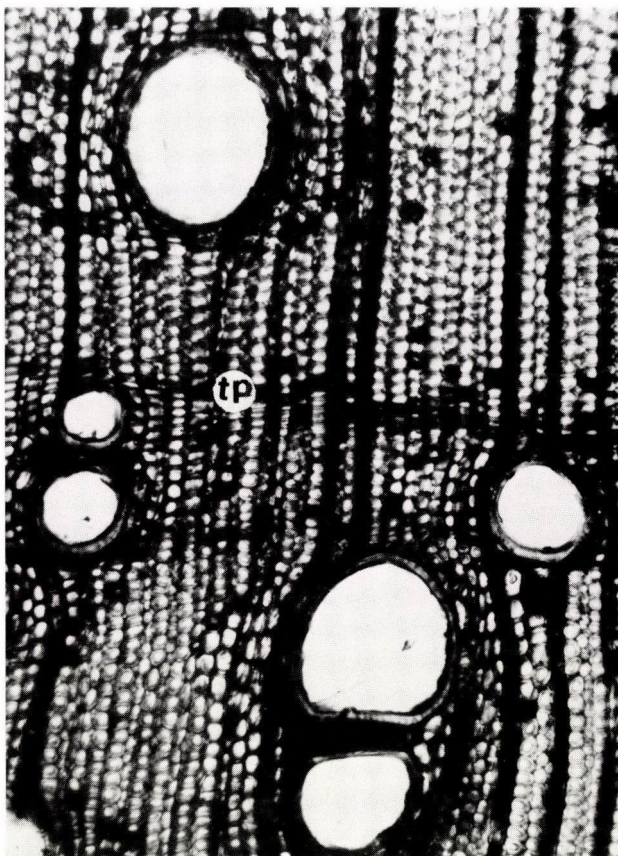


Fig. 4. Albizzia lebbeck (L.) Benth. Cross-section 120 x. Vessels, medullary rays, fibres. Contact-vasicentric and scanty aliform-confluent longitudinal parenchyma. Longitudinal parenchyma of terminal position (terminal longitudinal parenchyma = tp)



Fig. 5. *Albizzia lebbeck* (L.) Benth. Tangential longitudinal section 120 x. One- and two-cell wide medullary rays, vessel, longitudinal parenchyma and fibres. The medium size alternately set elongated bordered pits in the walls of vessels are clearly seen. Longitudinal parenchyma with calcium-oxalate crystals (crystal holder longitudinal parenchyma = cp)

times extremely elongated bordered pits in the walls. Simple perforation plate.

The medullary rays are 1-2-cell wide with homogeneous structure, 34.5-116.38-218.50 μm in height, 11.50-13.80-17.25 μm in width (Figs 5, 6).

Fibres arranged in radial rows. The fibre diameter is 9.30-16.65-23.25 μm , the wall thickness 1.16-2.44-4.65 μm . Septate fibres are not infrequent. The fibres end in smooth tips. The total length of fibres is 497.00-831.41-1065.0 μm . The cells of medullary rays often contain dark mastic material and diamond-shaped calcium oxalate crystals (METCALFE and CHALK 1950).

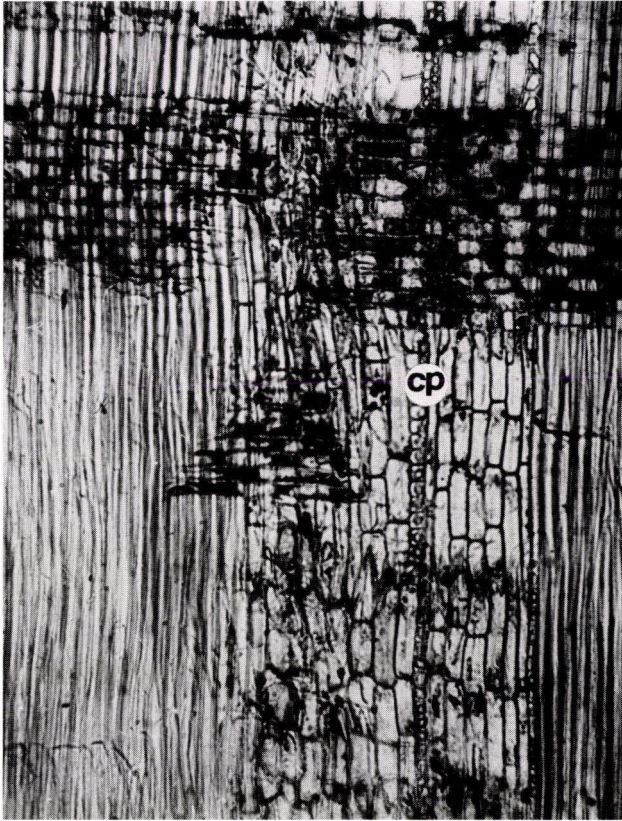


Fig. 6. *Albizzia lebbeck* (L.) Benth. Radial longitudinal section 120 x. Homogeneous medullary rays, longitudinal parenchyma, fibres. Dark mastic material in the medullary ray cells. Longitudinal parenchyma with calcium oxalate crystals (crystal holder longitudinal parenchyma = cp)

The tangential diameter of the longitudinal parenchyma cells is 13.95–18.14–23.25 μm . The height of cell is 55.80–82.03–139.50 μm . The crystal holder longitudinal parenchyma is frequent. Dark mastic material can be found in the cells.

Inga spuria H. et B. (Table 2, Figs 7--9)

Diffuse-porous wood. The bulk of wood is made up of relatively thin-walled, medium size fibres of polygonal shape. Aliform-confluent, seldom contact-vasicentric longitudinal parenchyma. Narrow medullary rays (Fig. 7).

The trachea are round or oval, solitary, sometimes in pairs; in the infrequent groups of 3-4 they are tangentially flattened. Their number is 8-17.2-35.0/mm². The tangential diameter is 37.20-109.83-167.40 μ m, the radial diameter 51.15-115.32-181.35 μ m. The vessel members are 172.50-287.04-379.50 μ m long, with medium size, bordered pits of alternate position in the walls. Simple perforation plate. Dark mastic material is seldom found in the vessels.

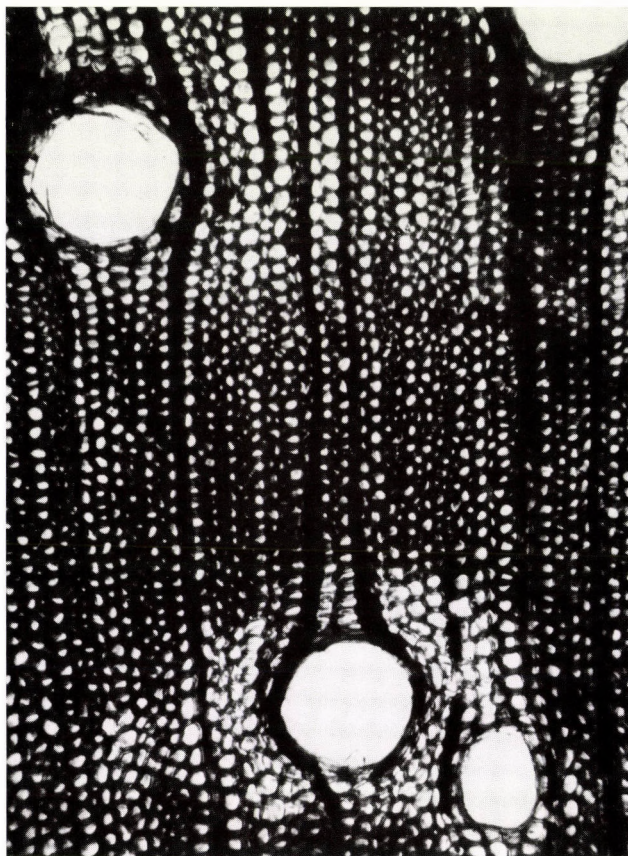


Fig. 7. Inga spuria H. et B. Cross-section 120 x. Vessels, medullary rays, fibres. Aliform-confluent longitudinal parenchyma



Fig. 8. *Inga spuria* H. et. B. Tangential longitudinal section 120 x. One- and two-cell wide medullary rays, vessel, longitudinal parenchyma and fibres. In the walls of vessels the alternately set medium size bordered pits are clearly seen. Septate crystal holder longitudinal parenchyma (cp)

The medullary rays are 1-2-, sometimes 3-cell wide, with homogeneous or seldom heterogeneous structure; 57.50-193.20-379.50 μm high and 8.62-16.96-28.75 μm wide (Figs 8, 9).

The fibres are arranged in radial rows. The fibre diameter is 9.30-15.43-23.25 μm , the wall thickness 1.16-1.97-3.48 μm . The total length of fibres is 568.00-764.83-1065.00 μm . The fibres generally end in smooth tips, but sometimes are serrate at one end. Septate fibres occur.

The tangential diameter of the longitudinal parenchyma cells is 13.95-18.60-23.25 μm . Their height is 41.85-94.95-144.15 μm . Septate crystal holder longitudinal parenchyma is frequent.



Fig. 9. *Inga spuria* H. et B. Radial longitudinal section 120 x. Homogeneous medullary rays, vessel, longitudinal parenchyma and fibres. In the medullary ray cells dark mastic material

The detailed anatomical characteristics of wood in the three tree species together with their measurements are contained in Tables 1 and 2.

Table 2

Anatomical features of the species examined

Wood elements	Features	<i>Inga spuria</i>
Trachea	arrangement	diffused, solitary and duplicate, rarely in radial groups of 3-4 members
	shape	roundish or oval, shaped in groups in tangential direction flattened
	tangential diameter	37.2-109.83-167.4 μm
	radial diameter	51.15-115.32-181.35 μm
	length of vessels	172.5-287.04-379.50 μm
	number per mm^2	8.0-17.2-35.0
	wall thickness	1.16-4.44-6.97 μm
	intervascular pitting	alternate, bordered
	perforate plate	simple
	content	rarely mastic material
Medullary rays	width	narrow
	number of cells	1-2, rarely 3
	classification	homogeneous, rarely heterogeneous
	height	57.5-193.2-379.5 μm
	width	8.62-16.96-28.75 μm
	content	mastic material
Fibres	arrangement	radial rows
	shape	polygonal
	full thickness	9.3-15.43-23.25 μm
	wall thickness	1.16-1.97-3.48 μm
	full length	568.0-764.83-1065.0 μm
	type of pitting	small, bordered
	other	septate fibre
Longitudinal parenchyma	arrangement	aliform-confluent, rarely contact-vasicentric
	diameter	13.95-18.60-23.25 μm
	height	41.85-94.95-144.15 μm
	number of cells	2-4
	content	calcium-oxalate crystal
	other	septad crystal-holder longitudinal parenchyma

Acacia glomerosa Benth. (Table 3, Figs 10–12)

Wood diffuse-porous. Bulk of wood consists mainly of thin-walled fibres of polygonal shape. Longitudinal parenchyma is contact-vasicentric and aliform-confluent. Medullary rays are narrow (Fig. 10).

Tracheae rounded or ovate, generally solitary, sometimes in pairs, rarely in 3-membered radial groups. Vessels are tangentially flattened. Their number is 8–11.2–21 per square mm. Their tangential diameter is 69.75–108.99–172.05 μm . The radial diameter is 69.75–114.39–139.50 μm . The vessel members are 69.0–235.17–345.0 μm long with alternately set medium sized elongated bordered pits in the walls. Perforation plate is simple.

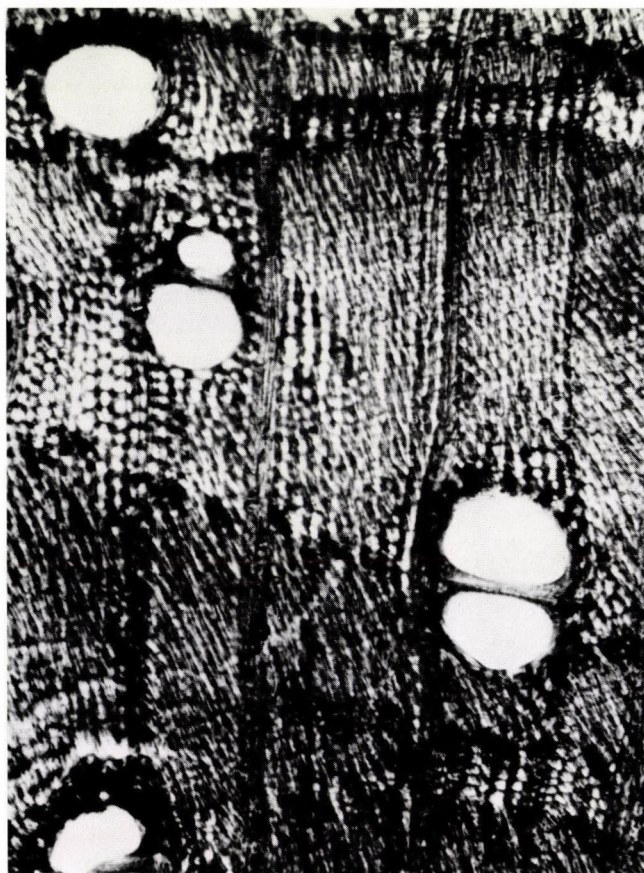


Fig. 10. Acacia glomerosa Benth. Cross-section 120x. Vessels, medullary rays, fibres. Contact-vasicentric and aliform-confluent longitudinal parenchyma



Fig. 11. *Acacia glomerosa* Benth. Tangential section 120 x. 1-2 and 3-cell wide medullary rays, longitudinal parenchyma and fibres. Ca-oxalate crystals in the longitudinal parenchyma

Medullary rays are usually 2-4-cell, rarely 1-cell wide with homogeneous or heterogeneous structure. They are 57.5-245.3-471.5 μm high and 11.5-24.8-46.0 μm wide (Figs 11-12).

Fibres arranged in radial rows, their diameter is 11.62-14.57-18.60 μm , wall thickness is 2.32-4.16-4.65 μm . The total length of the fibres is 497.0-768.22-1065.0 μm , ended in a long smooth tip.

Tangential diameter of the longitudinal parenchyma cells is 9.30-14.29-18.60 μm , their height is 46.50-94.62-162.75 μm . Crystal containing longitudinal parenchyma is not rare.



Fig. 12. Acacia glomerosa Benth. Radial section 120 x. Homogeneous medullary rays, vessels and fibres

Table 3
Anatomical features of the species examined

Wood elements	Features	<i>Acacia glomerosa</i>	<i>Acacia macracantha</i>
Trachea	arrangement	diffused, solitary and duplicate, rarely in radial groups 3 members	diffused, solitary and duplicate, rarely in radial groups 3-4 members
	shape	oval, roundish, shaped in groups in tangential direction flattened	oval, roundish, shaped in groups in tangential direction flattened
	tangential diam.	69.75-108.99-172.05 μm	55.8-88.53-144.15 μm
	radial diameter	69.75-114.39-139.5 μm	27.9-87.97-139.5 μm
	length of vessels	69.0-235.17-345.0 μm	92.0-185.15-253.0 μm
	number per mm^2	8-11.2-21	5-9.53-13
	wall thickness	4.65-6.16-9.30 μm	2.32-4.18-9.30 μm
	intervascular pitting	elongated bordered	elongated bordered
	perforate plate	simple	simple
	content	—	—
Medullary rays	width	narrow	narrow
	number of cells	2-4, rarely 1	1-2, rarely 3
	classification	homogeneous and heterogeneous	homogeneous
	height	57.5-245.3-471.5 μm	80.5-190.13-333.5 μm
	width	11.5-24.8-46.0 μm	11.5-16.44-23.0 μm
	content	—	—
Fibres	arrangement	radial rows	radial rows
	shape	polygonal	polygonal
	full thickness	11.62-14.57-18.60 μm	13.95-18.60-23.25 μm
	wall thickness	2.32-4.16-4.65 μm	0.93-2.69-4.65 μm
	full length	497.0-768.22-1065.0 μm	568.0-879.69-1207.0 μm
	type of pitting	small, bordered	small, bordered
	other	—	—
Longitudinal parenchyma	arrangement	contact-vasicentric and aliform-confluent	contact vasicentric
	diameter	9.30-14.29-18.60 μm	9.30-14.76-25.57 μm
	height	46.50-94.62-162.75 μm	37.2-91.48-162.75 μm
	number of cells	2-4	2-4
	content	—	—
	other	frequent septad crystal-holder longitudinal parenchyma	rarely septad crystal-holder longitudinal parenchyma

Acacia macracantha H. et B. (Table 3, Figs 13—15)

Wood diffuse-porous, its bulk is made up of polygonal-shaped thin-walled fibres. Longitudinal parenchyma is contact-vasicentric. Medullary rays are thin (Fig. 13).

Tracheae rounded or ovate, solitary or in pairs. Radial groups of 3-4 are infrequent. Vessels are tangentially flattened. Their number is 5.0-9.53-13.0 per square mm. Tangential diameter is 55.80-88.53-144.15 μm , radial diameter is 27.90-87.97-139.50 μm . Vessel members are 92.0-185.15-253.0 μm long with alternately set medium sized elongated bordered pits in the walls. Simple perforation plate.

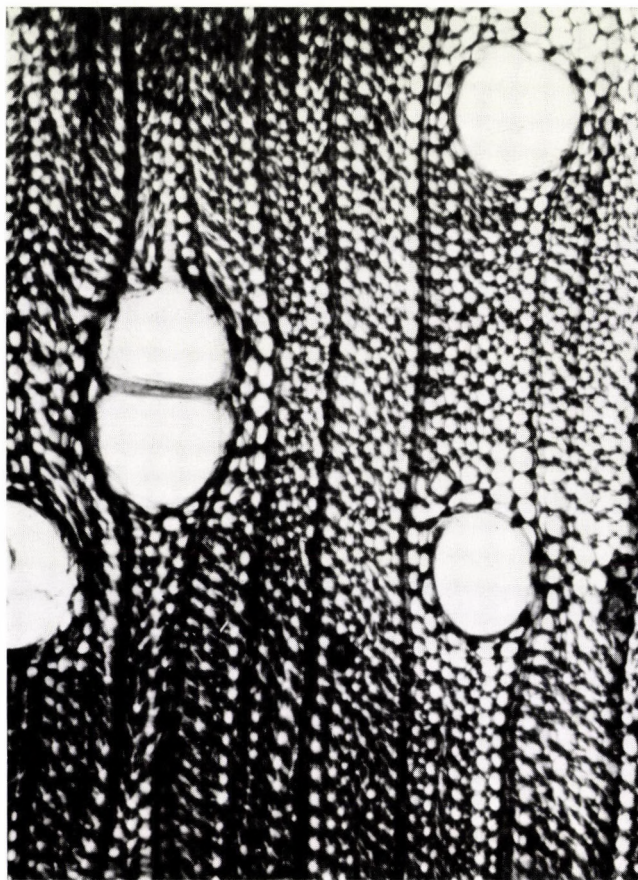


Fig. 13. Acacia macracantha H. et B. Cross-section 120 x. Vessels, medullary rays, fibres.
Contact-vasicentric longitudinal parenchyma

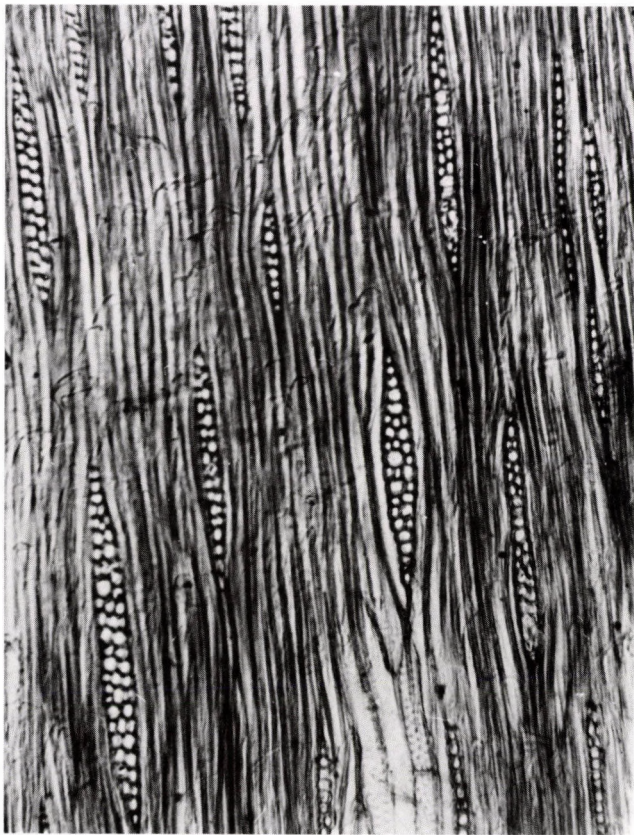


Fig. 14. *Acacia macracantha* H. et B. Tangential section 120 x. 1-2 and 3-cell wide medullary rays and fibres

Medullary rays are 1-2-cell, rarely 3-cell wide with homogeneous structure. They are 80.5-190.13-333.5 μm high and 11.5-16.44-23.0 μm wide (Figs 14-15).

Fibres arranged in radial rows. Their diameter is 13.95-18.60-23.25 μm , wall thickness is 0.93-2.69-4.65 μm . Fibres end in smooth tips, their total length is 568.0-819.69-1207.0 μm .

Tangential diameter of the longitudinal parenchyma cells is 9.30-14.76-25.57 μm . The height of cells is 37.20-91.48-162.75 μm . Sometimes crystal holder longitudinal parenchyma occurs.



Fig. 15. Acacia macracantha H. et B. Radial section 120 x. Homogeneous medullary rays, vessels and fibres. Longitudinal parenchyma with Ca-oxalate crystals

Acacia tamarindifolia (L.)Willd. (Table 4, Figs 16-18)

Diffuse-porous wood. Its bulk consists of polygonal shaped, relatively thick-walled fibres. Contact-vasicentric longitudinal parenchyma with apotracheal, marginal position. Medullary rays thin (Fig. 16).

Tracheae rounded or ovate, solitary rarely in pairs, sometimes forming 3-6 membered groups. They are tangentially flattened. Their number is 13-30-41 per square mm. Tangential diameter is 32.55-68.44-106.95 μm , radial diameter is 32.55-66.77-93.0 μm . Vessel members are 115.0-178.25-230.0 μm long with alternately set medium sized elongated bordered tips in the walls. Perforation plate simple. Sometimes in the vessels dark-coloured mastic material is found.

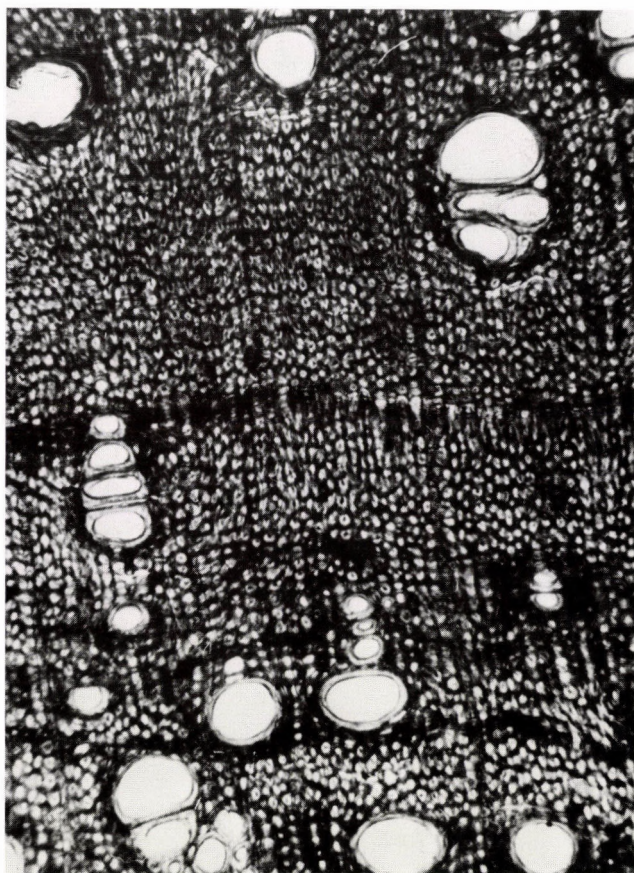


Fig. 16. Acacia tamarindifolia (L.)Willd. Cross-section 120x. Vessels, medullary rays, fibres.
Contact-vasicentric and apotracheal marginal longitudinal parenchyma



Fig. 17. *Acacia tamarindifolia* (L.) Willd. Tangential section 120 x. 1-2-cell wide medullary rays, fibres. Septate fibres with Ca-oxalate crystals

Medullary rays 1-2-cell wide with homogenous structure. Their height is 80.50-159.85-368.0 μm , width 11.50-12.26-23.0 μm (Figs 17-18).

Fibres arranged in radial rows, their diameter is 9.30-13.02-08.60 μm ; wall thickness 3.72-4.58-4.65 μm ; total length 426.0-611.31-994.0 μm . They end in a smooth tip, sometimes it is branched or serrate-margined on one end. Crystal holding septate fibres also occur.

Tangential diameters of longitudinal parenchyma cells are 6.97-10.23-13.95 μm their height is 20.29-41.94-55.80 μm .



Fig. 18. *Acacia tamarindifolia* (L.) Willd. Radial section 120 x. Homogeneous medullary rays, vessels, fibres. Septate fibres with Ca-oxalate crystals

Table 4
Anatomical features of the species examined

Wood elements	Features	<i>Acacia tamarindifolia</i>
Trachea	arrangement	diffused, solitary and duplicate, rarely in radial groups of 3-6 members
	shape	roundish or oval, shaped in groups in tangential direction flattened
	tangential diameter	32.55-68.44-106.95 μm
	radial diameter	32.55-66.77-93.00 μm
	length of vessels	115.00-178.25-230.00 μm
	number per mm^2	13-30-41
	wall thickness	2.32-4.85-9.30 μm
	intervascular pitting	alternate, bordered
	perforate plate	simple
	content	rarely mastic material
Medullary rays	width	narrow
	number of cells	1-2
	classification	homogeneous
	height	80.5-159.85-368.0 μm
	width	11.5-12.26-23.00 μm
	content	—
Fibres	arrangement	radial rows
	shape	polygonal
	full thickness	9.3-13.02-18.6 μm
	wall thickness	3.72-4.58-4.65 μm
	full length	426.0-611.31-994.0 μm
	type of pitting	small, bordered
	other	septate fibre, crystal-holder
Longitudinal parenchyma	arrangement	contact-vasicentric, apotracheal marginal
	diameter	6.97-10.23-13.95 μm
	height	20.92-41.94-55.80 μm
	number of cells	2-4
	content	—
	other	—

Mimosa arenosa (Willd.)Poir. (Table 5, Figs 19-21)

Diffuse-porous wood. The mass of the wood is given by rather thin-walled polygonal fibres. Contact vasicentric longitudinal parenchyma. Narrow medullary rays (Fig. 1).

Tracheae oval or round, set in singles, sometimes in twos. Radial groups of 3 are rare. Here the vessels are tangentially flattened; they are 7-14, 5-21/mm² in number. The tangential diameter is 60.4-107.9-172.0 μ m, the radial diameter 55.8-85.3-139.5 μ m. The vessel members are 57.5-133.4-207.0 μ m long, with alternately set small, elongate bordered pits on the walls. Perforation plate simple.

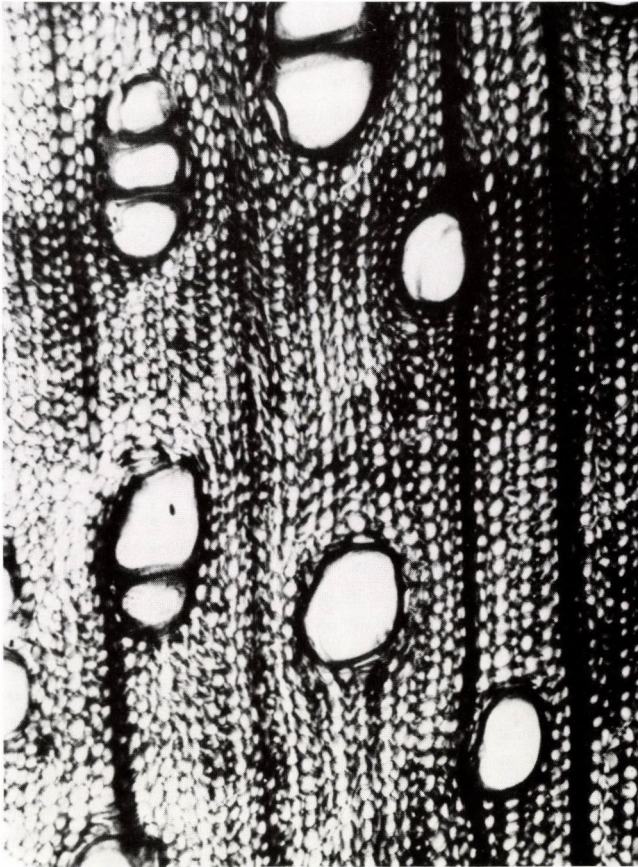


Fig. 19. Mimosa arenosa (Willd.)Poir. Cross-section 120x. Vessels, medullary rays, fibres.
Contact-vasicentric longitudinal parenchyma



Fig. 20. *Mimosa arenosa* (Willd.)Poir. Tangential longitudinal section 120 x. One-two-cell wide medullary rays, vessel, longitudinal parenchyma and fibres. Septate crystal-holder longitudinal parenchyma with calcium oxalate crystals. sp = septate crystal holder longitudinal parenchyma

The medullary rays are 1-2-cell wide with heterogeneous structure. Height 83.7-137.5-195.3 μm (Figs 20 and 21), width 9.3-18.04-23.25 μm . The septate crystal-holder longitudinal parenchyma with calcium oxalate crystals is clearly seen in the longitudinal sections.

Fibres arranged in radial rows. Diameter 13.8-17.9-23.0 μm ; wall thickness a constant 2.3 μm . Total length of fibres 568.0-758.2-852.0 μm . The fibres end in smooth tips.



Fig. 21. *Mimosa arenosa* (Willd.)Poir. Radial longitudinal section 120 x. Heterogeneous medullary rays, vessel, longitudinal parenchyma and fibres. Septate crystal holder longitudinal parenchyma with calcium oxalate crystals. sp = septate crystal holder longitudinal parenchyma

Table 5
Anatomical features of the species examined

Wood elements	Features	<i>Mimosa arenosa</i>	<i>Mimosa pigra</i>
Trachea	arrangement	diffused, solitary and duplicate, rarely in radial groups of 3 members	diffused, solitary and duplicate, rarely in radial groups of 3 members
	shape	oval or rounded, shaped in groups tangential direction flattened	rounded or oval, shaped in groups tangential direction flattened
	tangential diam.	60.4-107.9-172.0 μm	46.5-78.58-111.6 μm
	radial diameter	55.8-85.3-139.5 μm	37.2-86.3-139.5 μm
	length of vessels	57.5-133.4-207.0 μm	80.5-194.35-299.0 μm
	number per mm^2	7.0-14.52-21.0	8.0-15.52-28.0
	wall thickness	4.65-5.48-9.30 μm	2.32-4.92-9.30 μm
	intervascular pitting	bordered, small, alternate elongated	bordered, alternate
	perforate plate	simple	simple
	content	—	—
Medullary rays	width	narrow	narrow
	number of cells	1-2	1
	classification	heterogeneous	heterogeneous
	height	83.7-137.54-195.3 μm	106.9-196.88-325.5 μm
	width	9.3-18.04-23.25 μm	9.3-13.85-23.25 μm
	content	—	mastic material
Fibres	arrangement	radial rows	radial rows
	shape	polygonal	polygonal
	full thickness	13.8-17.98-23.0 μm	11.5-18.26-23.0 μm
	wall thickness	2.3 μm	2.3 μm
	full length	568.0-758.28-852.0 μm	568.0-761.12-1136.0 μm
	type of pitting	small, bordered	small, bordered
	other	—	—
Longitudinal parenchyma	arrangement	contact-vasicentric	contact-vasicentric
	diameter	9.3-15.34-23.25 μm	9.3-15.71-23.25 μm
	height	41.8-89.0-144.15 μm	27.9-66.03-125.55 μm
	number of cells	2-3-4	2-3-4
	content	—	—
	other	septate crystal-holder longitudinal parenchyma	—

Mimosa pigra L. (Table 5, Figs 22--24)

Diffuse-porous wood. The mass of the wood is formed by rather thin-walled polygonal fibres. Contact vasicentric longitudinal parenchyma, narrow medullary rays (Fig. 22).

Tracheae round or oval, arranged in singles, sometimes in twos. Radial groups of 3 members are rare. Here the vessels are tangentially flattened; they are $8-15.5-28.0/\text{mm}^2$ in number. Tangential diameter $46.5-78.5-111.6\ \mu\text{m}$, radial diameter $37.2-86.3-139.5\ \mu\text{m}$. The members of vessels are $80.5-194.3-299.0\ \mu\text{m}$ in length, with alternately set, medium size, ornate bordered pits on the walls. Perforation plate simple. As seen in Fig. 22, the cambium filled up the injury of bark with parenchyma cells.



Fig. 22. Mimosa pigra L. Cross-section 120 x. Vessels, medullary rays, fibres. Contact-vasicentric longitudinal parenchyma. Xylem overgrowing the injury of bark. Injury of bark filled up with parenchyma



Fig. 23. *Mimosa pigra* L. Tangential longitudinal section 120x. Vessel, longitudinal parenchyma, fibre. One-cell wide medullary rays. In the cells of medullary ray and longitudinal parenchyma black mastic matter

The medullary rays are one-cell wide, of heterogeneous structure. Height 106.9-196.8-325.5 μm , width 9.3-13.8-23.2 μm (Figs 23 and 24). The medullary ray- and longitudinal parenchyma cells contain black mastic material.

Fibres arranged in radial rows; diameter 11.5-18.2-23.0 μm , wall thickness constant 2.3 μm . Total length of fibres 568.0-761.1-1136.0 μm . Fibres end in smooth tips.

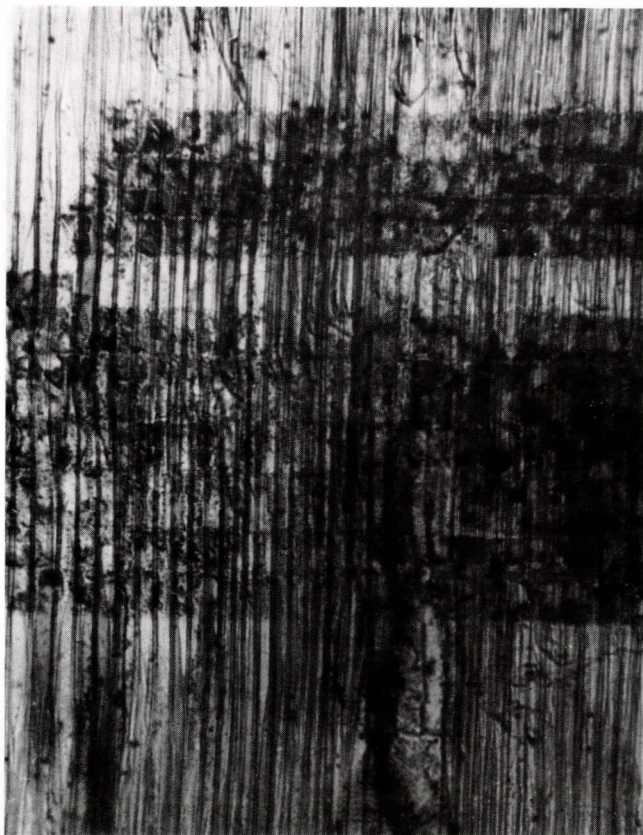


Fig. 24. Mimosa pigra L. Radial section 120 x. Heterogeneous medullary rays. Fibres and longitudinal parenchyma

Enterolobium cyclocarpum (Jacq.)Griseb. (Table 6, Figs 25—27)

Diffuse-porous wood. The mass of wood is composed of polygonal fibres with medium thick walls. The longitudinal parenchyma is paratracheal, contact vasicentric. Narrow medullary rays (Fig. 25). The cross-section of the wood clearly shows the growth zones and their borders.

Tracheae oval, generally singular, though radial groups of 2-4 often occur. In the radial groups the tracheae are tangentially flattened. They are $4-7.6-13/\text{mm}^2$ in number. Tangential diameter $51.1-130.1-172.0\ \mu\text{m}$, radial diameter $37.2-108.3-148.8\ \mu\text{m}$. The vessel members are $69.0-211.3-414.0\ \mu\text{m}$ in length, with alternately set, medium size, highly elongated bordered pits on the walls. Simple perforation plate.

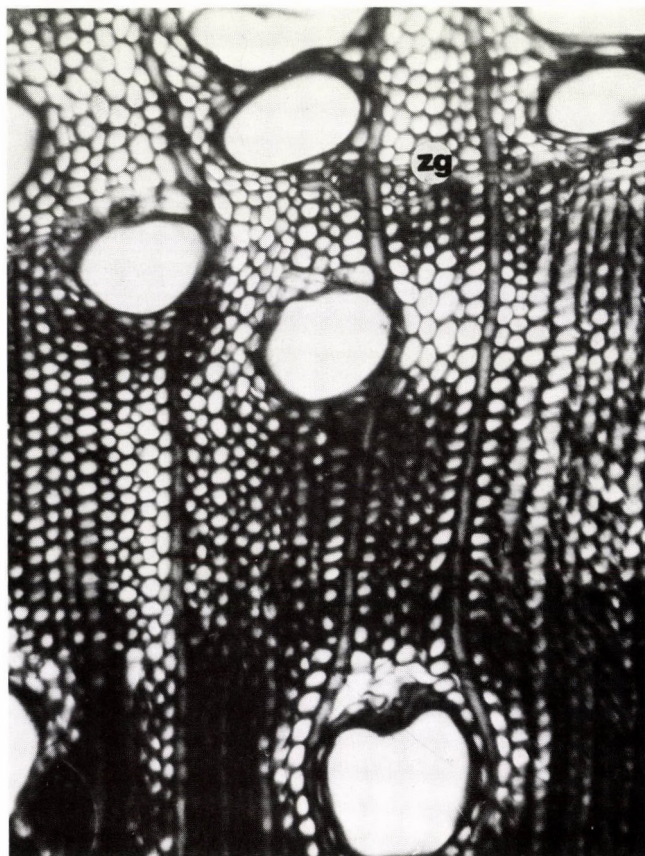


Fig. 25. Enterolobium cyclocarpum (Jacq.)Griseb. Cross-section 120 x. Vessels, medullary rays, fibres. Paratracheal contact-vasicentric longitudinal parenchyma. The border of the growth zone is clearly seen. zg = zone of growth



Fig. 26. *Enterolobium cyclocarpum* (Jacq.) Griseb. Tangential longitudinal section 120x. One-two-cell wide medullary rays. Vessel, longitudinal parenchyma, fibre. Septate crystal holder longitudinal parenchyma with calcium oxalate crystals

Medullary rays one- or sometimes two-cell wide, with homogeneous structure. Height 125.5-255.5-427.8 μm ; width 9.3-14.97-23.2 μm (Figs 26 and 27). Septate crystal-holder longitudinal parenchyma with calcium oxalate crystals occurs.

Fibres arranged in radial rows. Fibre diameter 16.1-20.6-27.6 μm ; wall thickness 2.3-2.85-6.9 μm . Total length of fibres 639.0-861.9-1207.0 μm . Fibres end in smooth, short tips.

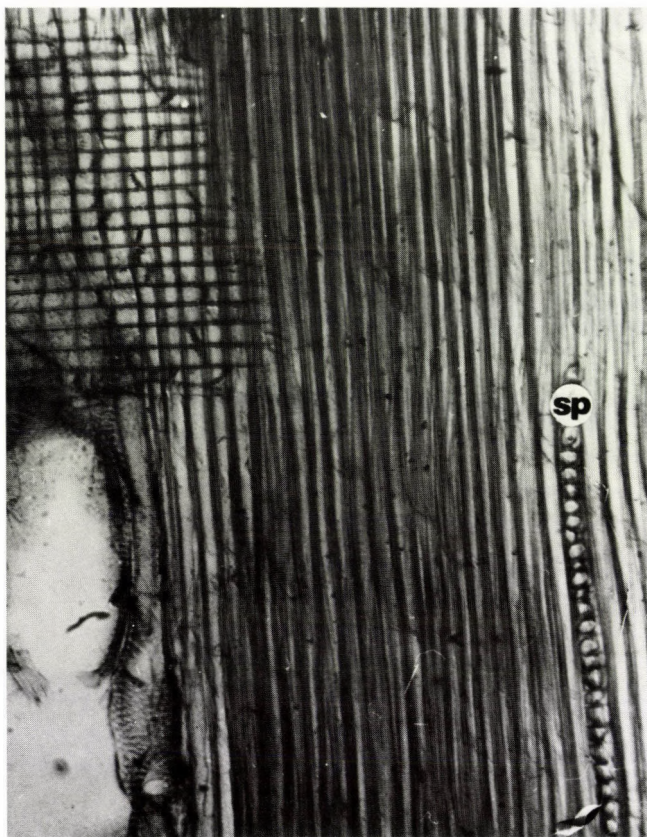


Fig. 27. Enterolobium cyclocarpum (Jacq.) Griseb. Radial longitudinal section 120 x. Homogeneous medullary ray. Vessel, longitudinal parenchyma, fibre. Longitudinal parenchyma, septate, crystal-holder, with calcium oxalate crystals. sp = septate crystal-holder longitudinal parenchyma

Table 6
Anatomical features of the species examined

Wood elements	Features	<i>Enterolobium cyclocarpum</i>	<i>Leucaena latisiliqua</i>
Trachea	arrangement	diffused, solitary, rarely in radial groups of 2-4 members	diffused, solitary and duplicate, rarely in radial groups of 3-6 members
	shape	oval, shaped in groups tangential direction flattened	oval, shaped in groups tangential direction flattened
	tangential diam.	51.15-130.1-172.05 μm	60.45-114.85-167.40 μm
	radial diameter	37.2-108.43-148.8 μm	46.5-92.72-144.15 μm
	length of vessels	69.0-211.37-414.0 μm	80.5-163.76-299.0 μm
	number per mm^2	4-7.68-13	2-4.04-9
	wall thickness	4.65-7.68-13.95 μm	4.65-5.67-9.3 μm
	intervascular pitting	bordered, elongated, alternate	bordered, alternate
	perforate plate	simple	simple
	content	—	—
Medullary rays	width	narrow	narrow
	number of cells	1, rarely 2	1-2
	classification	homogeneous	homogeneous
	height	125.5-255.56-427.8 μm	65.1-135.08-209.25 μm
	width	9.3-14.97-23.25 μm	9.3-15.34-27.9 μm
	content	—	—
Fibres	arrangement	radial rows	radial rows
	shape	polygonal	polygonal
	full thickness	16.1-20.65-27.6 μm	6.24-8.73-12.48 μm
	wall thickness	2.3-2.85-6.9 μm	0.78-1.23-1.56 μm
	full length	639.0-861.94-1207.0 μm	710.0-1012.46-1349.0 μm
	type of pitting	small, bordered	small, bordered
	other	—	—
Longitudinal parenchyma	arrangement	paratracheal, contact- vasicentric	scattered-diffuse, contact- vasicentric
	diameter	9.3-17.01-23.25 μm	13.95-17.94-27.9 μm
	height	27.9-96.62-162.7 μm	55.8-79.22-116.25 μm
	number of cells	2-4-6	2-4
	content	—	—
	other	septate crystal-holder longitudinal parenchyma	septate crystal-holder longitudinal parenchyma

Leucaena latisiliqua (L.) Gillis. (Table 6, Figs 28--30)

Diffuse-porous wood. The mass of wood is composed of polygonal, thin-walled fibres. The longitudinal parenchyma is scattered-diffuse and contact vasicentric (METCALFE and CHALK 1950). Narrow medullary rays (Fig. 28).

Tracheae oval, set in singles, sometimes in twos, seldom in radial groups of 3-6. In the latter the tracheae are tangentially flattened, $2-4-9/\text{mm}^2$ in number. Tangential diameter $60.4-114.8-167.4\ \mu\text{m}$; radial diameter $46.5-92.7-144.1\ \mu\text{m}$. Vessel members $80.5-163.7-299.0\ \mu\text{m}$ in length, with alternately set, medium size pits on the walls. Simple perforation plate.

Medullary rays 1-2-cell wide, of homogeneous structure. Height $65.1-135.0-209.2\ \mu\text{m}$; width $9.3-15.3-27.9\ \mu\text{m}$. In the scattered-diffuse longi-

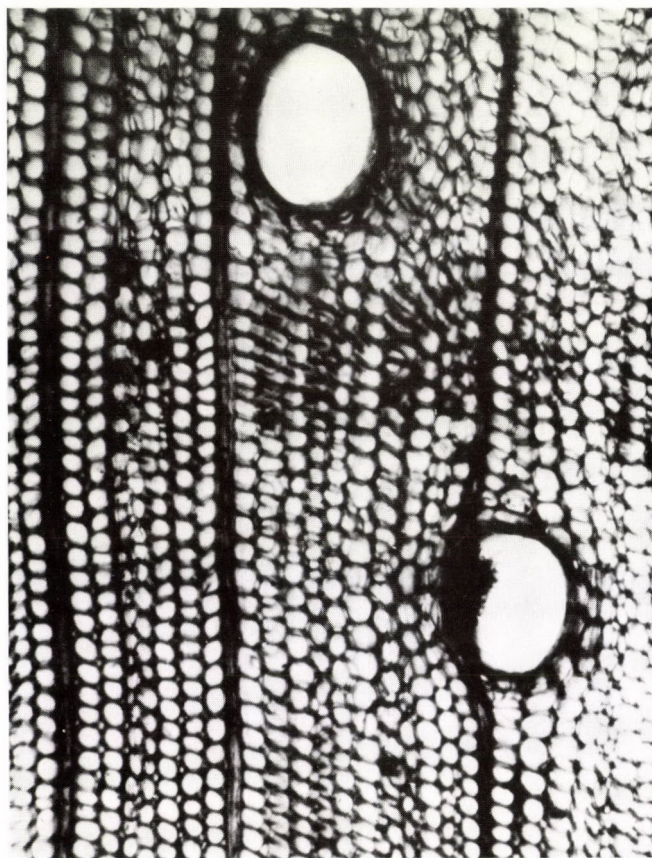


Fig. 28. Leucaena latisiliqua (L.) Gillis. Cross-section 120x. Vessels, medullary rays, fibres. Contact-vasicentric and scattered-diffuse longitudinal parenchyma



Fig. 29. *Leucaena latisiliqua* (L.) Gillis. Tangential longitudinal section 120 x. One-two-cell wide medullary rays. Longitudinal parenchyma, fibres. In the longitudinal parenchyma calcium oxalate crystals

tudinal parenchyma there are many septate crystal-holders with calcium oxalate crystals (Figs 29 and 30).

Fibres arranged in radial rows. Fibre diameter 6.2-8.7-12.4 μm ; wall thickness 0.78-1.23-1.56 μm . Total length of fibres 710.0-1012.4-1349.9 μm . Fibres end in smooth, short tips.

Detailed anatomical characteristics and measurements of xylem for the four tree species are contained in Tables 5 and 6.

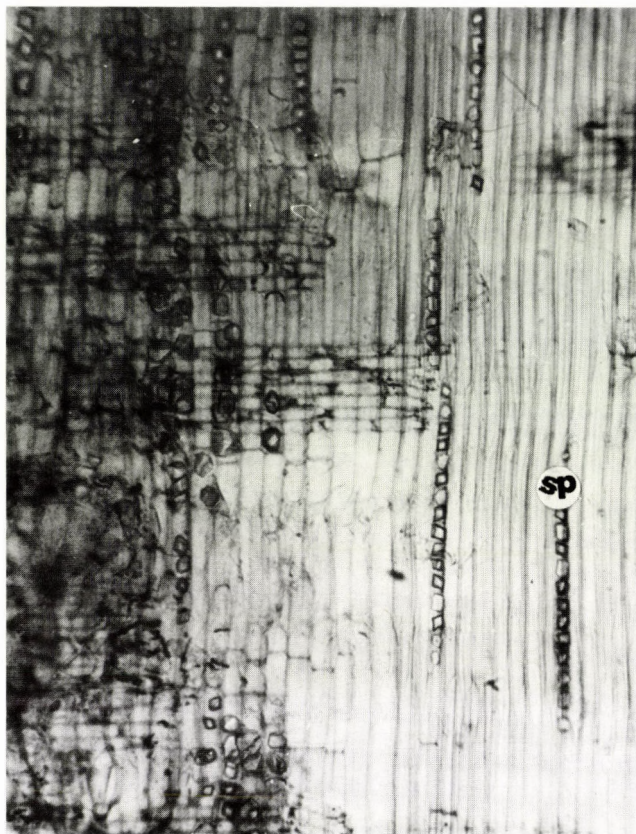


Fig. 30. *Leucaena latisiliqua* (L.) Gillis. Radial longitudinal section 120 x. Homogeneous medullary rays. Longitudinal parenchyma. Many septate crystal-holder longitudinal parenchyma with calcium oxalate crystals. sp = septate crystal-holder longitudinal parenchyma

Pithecellobium ligustrinum (Jacq.)Klotzh. (Table 7, Figs 31-34)

Diffuse-porous wood. The mass of the xylem is made up by polygonal fibres with thin walls. The longitudinal parenchyma is aliform-confluent. The medullary rays are narrow (Fig. 31).

Tracheae round or oval, generally singly, sometimes in twos. Radial groups of 3 are rare. Here the vessels are tangentially flattened. They are $5-8.8-15/\text{mm}^2$ in number. Tangential diameter $41.8-88.9-120.9\ \mu\text{m}$. Radial diameter $41.8-77.6-120.9\ \mu\text{m}$. Length of vessel members $46.0-125.8-230.0\ \mu\text{m}$; medium size, elongate bordered pits of alternate position on the walls. Simple perforation plate. As clearly seen in Fig. 32 the cambium has filled the injury of cortex with parenchyma cells. In the vessels, medullary ray- and longitudinal parenchyma cells black mastic can be found (Fig. 32).

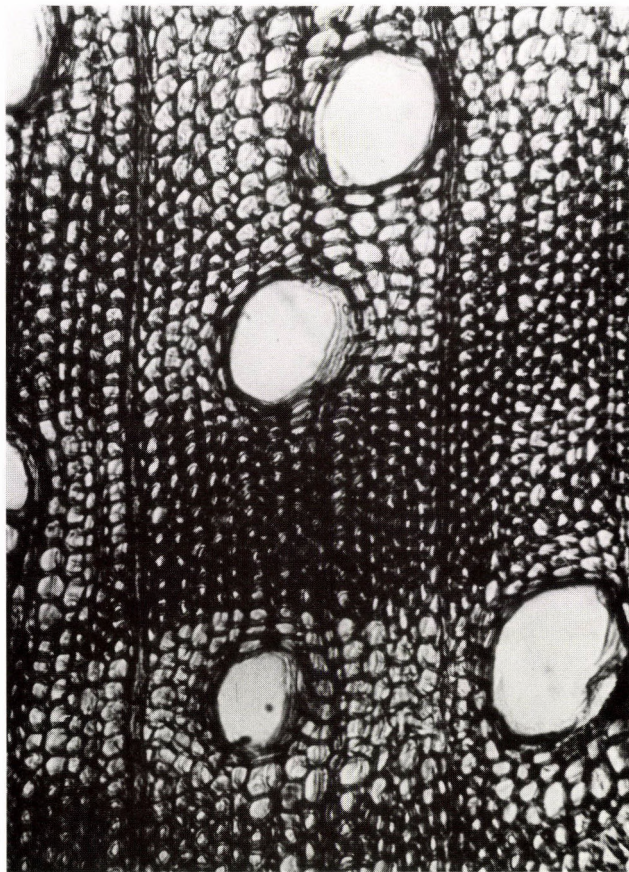


Fig. 31. Pithecellobium ligustrinum (Jacq.)Klotzh. Cross-section 120 x. Vessels, medullary rays, fibres. Aliform-confluent longitudinal parenchyma

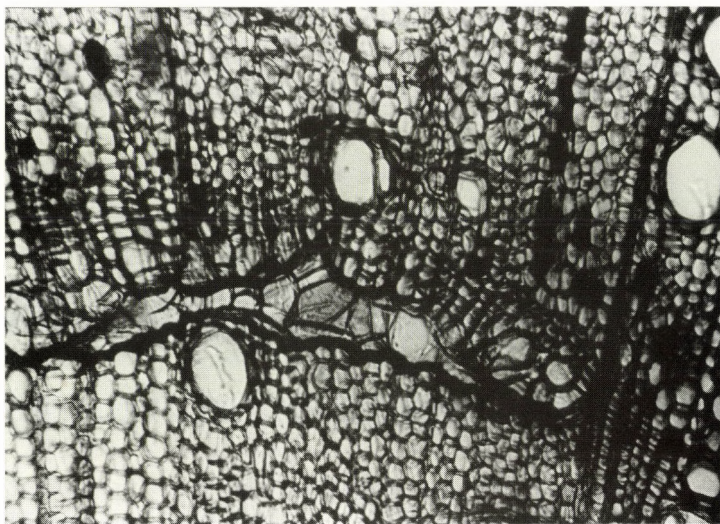


Fig. 32. *Pithecellobium ligustrinum* (Jacq.) Klotzh. Cross-section 120 x. Xylem growing over an injury of cortex. Cortical injury filled with parenchyma. In the vessels, medullary ray cells and longitudinal parenchyma cells black mastic



Fig. 33. *Pithecellobium ligustrinum* (Jacq.) Klotzh. Tangential longitudinal section 120 x. One- to two-cell wide medullary rays, vessel, longitudinal parenchyma and fibres



Fig. 34. *Pithecellobium ligustrinum* (Jacq.)Klotzh. Radial longitudinal section 120 x. Homogeneous medullary rays, longitudinal parenchyma and fibres

Medullary rays 1-2-cell wide, of homogeneous structure. Height 92.0-191.6-333.5 μm ; width 11.5-12.9-23.0 μm (Figs 33-34).

Fibres arranged in radial rows. Fibre diameter 18.6-27.5-41.8 μm . Wall thickness 4.65-6.32-9.30 μm . Full length of fibres 426.0-691.0-1065.0 μm . Fibres end in short, smooth tips.



Fig. 32. *Pithecellobium ligustrinum* (Jacq.)Klotzh. Cross-section 120 x. Xylem growing over an injury of cortex. Cortical injury filled with parenchyma. In the vessels, medullary ray cells and longitudinal parenchyma cells black mastic

Fig. 33. *Pithecellobium ligustrinum* (Jacq.)Klotzh. Tangential longitudinal section 120 x. One- to two-cell wide medullary rays, vessel, longitudinal parenchyma and fibres

Table 7

Anatomical features of the species examined

Wood elements	Features	<i>Pithecellobium ligustrinum</i>	<i>Pithecellobium tortum</i>
Trachea	arrangement	diffused, solitary and duplicate, rarely in radial groups of 3 members	diffused, solitary and duplicate, rarely in radial groups of 3 members
	shape	oval or rounded, shaped in groups tangential direction flattened	oval or rounded, shaped in groups tangential direction flattened
	tangential diam.	41.8-88.9-120.9 μm	37.2-59.1-79.0 μm
	radial diameter	41.8-77.6-120.9 μm	41.8-69.3-97.6 μm
	length of vessels	46.0-125.8-230.0 μm	34.5-105.8-184.0 μm
	number per mm^2	5-8.8-15	16-20.5-25
	wall thickness	9.3-10.9-18.6 μm	2.32-3.86-5.58 μm
	intervascular pitting	bordered, alternate	bordered, alternate
	perforate plate	simple	simple
	content	—	—
Medullary rays	width	narrow	narrow
	number of cells	1-2	1, rarely 2
	classification	homogeneous	homogeneous
	height	92.0-191.6-333.5 μm	11.5-224.4-540.5 μm
	width	11.5-12.9-23.0 μm	11.5-12.4-23.0 μm
	content	—	—
Fibres	arrangement	radial rows	radial rows
	shape	polygonal	polygonal
	full thickness	18.6-27.5-41.8 μm	18.6-22.6-27.9 μm
	wall thickness	4.65-6.32-9.30 μm	0.93-1.84-3.72 μm
	full length	426.0-691.0-1065.0 μm	426.0-627.6-994.0 μm
	type of pitting	small, bordered	small, bordered
	other	—	—
Longitudinal parenchyma	arrangement	aliform-confluent	scattered-diffuse and contact-vasicentric
	diameter	9.3-20.6-32.5 μm	9.3-12.8-23.2 μm
	height	13.9-74.5-186.0 μm	18.6-55.3-125.5 μm
	number of cells	2-4	2-4
	content	—	calcium-oxalate crystal, septate crystal-holder
	other	—	longitudinal parenchyma

Pithecellobium tortum Mart. (Table 1, Figs 35-37)

Diffuse-porous wood. The mass of xylem is made up by thin-walled polygonal fibres. Longitudinal parenchyma scattered-diffuse and contact-vasicentric. Medullary rays narrow. The growth zones are clearly visible (Fig. 35).

Tracheae round or oval, singly, often in twos. Radial groups of 3 are not rare. Here the vessels are tangentially flattened. They are 16-20.5-25/mm² in number. Tangential diameter: 37.20-59.14-79.05 μ m. Radial diameter: 41.85-69.37-97.65 μ m. The vessel members are 34.5-105.8-184.0 μ m long, with alternately set elongate bordered pits of medium size on the walls. Simple perforation plate.

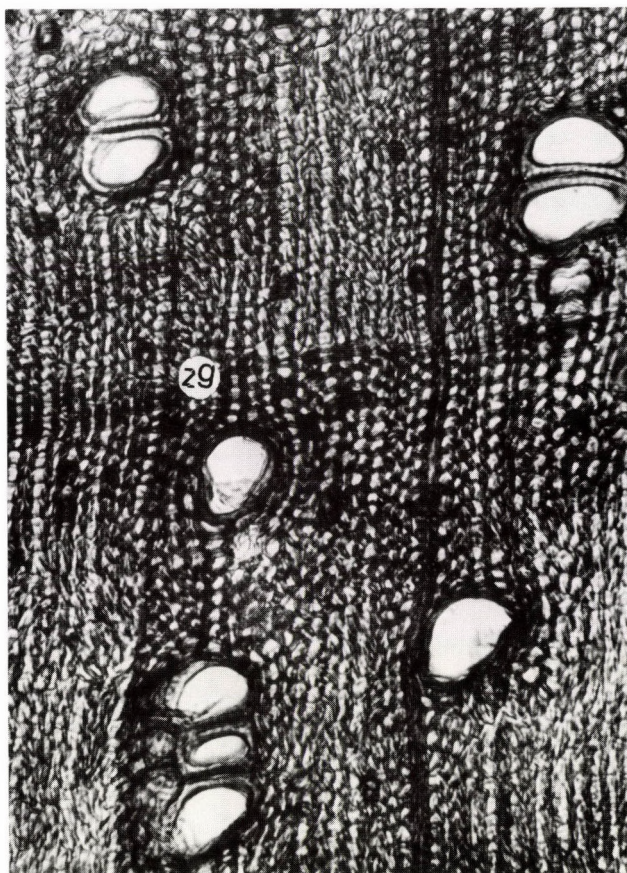


Fig. 35. Pithecellobium tortum Mart. Cross-section 120 x. Vessels, medullary rays, fibres. Scattered-diffuse and contact-vasicentric longitudinal parenchyma. Zone of growth (zg) clearly seen



Fig. 36. *Pithecellobium tortum* Mart. Tangential longitudinal section 120 x. One-, seldom two-cell wide medullary rays. Vessel, longitudinal parenchyma and fibres. Septate crystal-holder longitudinal parenchyma (sp) with calcium oxalate crystals

Medullary rays 1-, seldom 2-cell wide with homogeneous structure. Height: 11.5-224.4-540.5 μm . Width: 11.5-12.4-23.0 μm (Figs 36-37).

Fibres arranged in radial rows. Fibre diameter: 18.6-22.6-27.9 μm . Wall thickness: 0.93-1.84-3.72 μm . Total length of fibres: 426.0-627.6-994.0 μm . They end in smooth tips. Septate crystal-holder longitudinal parenchyma with polygonal calcium oxalate crystals frequently occurs in the xylem (Fig. 36).



Fig. 37. Pithecellobium tortum Mart. Radial longitudinal section 120 x. Heterogeneous medullary rays, vessel and fibres

Prosopis juliflora DC. (Table 8, Figs 38—40)

Diffuse-porous wood. The mass of the xylem is made up by polygonal fibres with thicker walls. Contact-vasicentric longitudinal parenchyma, narrow medullary rays (Fig. 37).

Tracheae round or oval, singly, though often in twos. Radial groups of 3 are not rare. In such cases the vessels are tangentially flattened. The vessels are $4-8.9-15/\text{mm}^2$ in number, and are of large size. Tangential diameter: $27.9-92.5-162.7\ \mu\text{m}$; radial diameter: $46.5-96.1-162.7\ \mu\text{m}$. The vessel members are $46.0-123.9-241.5\ \mu\text{m}$ long, with alternately set elongate bordered pits of medium size found on the walls in large numbers.

Medullary rays 1-2-3-cell wide, with homogeneous, sometimes heterogeneous structure. Height: $172.5-305.9-437.0\ \mu\text{m}$; width: $11.5-25.1-34.5\ \mu\text{m}$ (Figs 38—39).

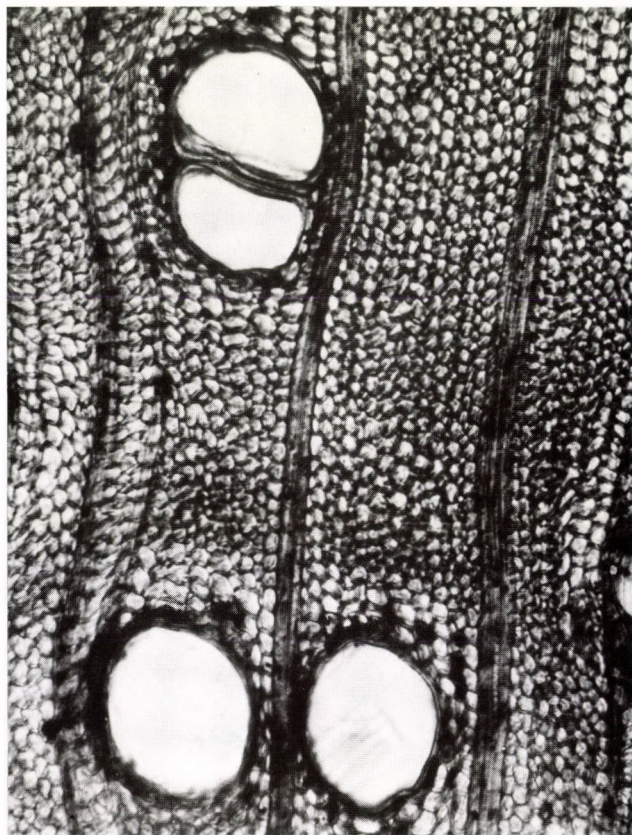


Fig. 38. Prosopis juliflora DC. Cross-section 120 x. Vessels, medullary rays, fibres. Contact-vasicentric longitudinal parenchyma

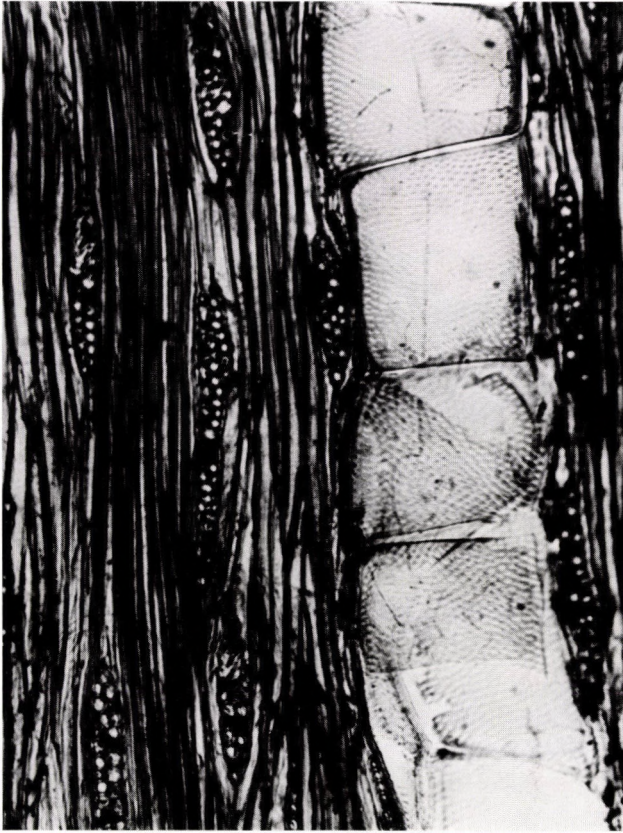


Fig. 39. *Prosopis juliflora* DC. Tangential longitudinal section 120 x. One-, two- and three-cell wide medullary rays. Vessel, longitudinal parenchyma and fibres

Fibres arranged in radial rows. Fibre diameter: 13.9-20.7-27.9 μm ; wall thickness: 4.65-5.02-9.30 μm ; total length of fibres: 497.0-748.3-1136.0 μm . Fibres end in short smooth tips. Septate crystal-holder longitudinal parenchyma with polygonal calcium oxalate crystals is not infrequent in the xylem (Fig. 40).



Fig. 40. Prosopis juliflora DC. Radial longitudinal section 120 x. Homogeneous medullary rays, fibres. Septate crystal-holder longitudinal parenchyma (sp)

Table 8
Anatomical features of the species examined

Wood elements	Features	<i>Prosopis juliflora</i>	<i>Piptadenia flava</i>
Trachea	arrangement	diffused, solitary and duplicate, rarely in radial groups of 3 members	diffused, solitary and duplicate, rarely in radial groups of 3-4 members
	shape	oval or rounded, shaped in groups tangential direction flattened	oval or rounded, shaped in groups tangential direction flattened
	tangential diam.	27.9-92.5-162.7 μm	27.9-61.9-97.5 μm
	radial diameter	46.5-96.1-162.7 μm	37.2-66.4-93.0 μm
	length of vessels	46.0-123.9-241.5 μm	57.5-140.3-230.0 μm
	number per mm^2	4-8.9-15	7-9.8-16
	wall thickness	9.3-10.1-13.9 μm	2.3-4.9-9.3 μm
	intervascular pitting	alternate, bordered	alternate, bordered
	perforate plate	simple	simple
	content	—	—
Medullary rays	width	narrow	narrow
	number of cells	1-2-3	1-2, rarely 3
	classification	homogeneous, rarely heterogeneous	heterogeneous
	height	172.5-305.9-437.0 μm	149.5-236.9-368.0 μm
	width	11.5-25.1-34.5 μm	11.5-21.3-34.5 μm
	content	—	—
Fibres	arrangement	radial rows	radial rows
	shape	polygonal	polygonal
	full thickness	13.9-20.7-27.9 μm	11.6-16.7-20.9 μm
	wall thickness	4.6-5.0-9.3 μm	2.3-3.4-4.6 μm
	full length	497.0-748.3-1136.0 μm	568.0-681.6-923.0 μm
	type of pitting	small, bordered	small, bordered
	other	—	—
Longitudinal parenchyma	arrangement	contact-vasicentric	contact-vasicentric
	diameter	9.3-17.4-23.2 μm	9.3-15.6-23.2 μm
	height	9.3-67.8-144.1 μm	32.5-62.1-144.1 μm
	number of cells	2-4	2-4
	content	calcium oxalate crystal	calcium oxalate crystal
	other	septate crystal-holder longitudinal parenchyma	septate crystal-holder longitudinal parenchyma

Piptadenia flava (Spreng.)Benth. (Table 8, Figs 41—43)

Diffuse-porous wood. The mass of the xylem is made up by polygonal fibres with medium thick walls. Contact-vasicentric longitudinal parenchyma; narrow medullary rays (Fig. 41). Tracheae in singles or in radial groups of 2-4. The single tracheae are round or oval, while those in groups are tangentially flattened. They are $7-9.8-16/\text{mm}^2$ in number. Tangential diameter: $27.9-61.9-97.6\text{ }\mu\text{m}$; radial diameter: $37.2-66.4-93.0\text{ }\mu\text{m}$. The vessel members are $57.5-140.3-230\text{ }\mu\text{m}$ long, with a large number of alternately set elongate bordered pits of medium size on the walls.

Medullary rays are 1-2-, seldom 3-cell wide, with heterogeneous structure. Height: $149.5-236.9-368.0\text{ }\mu\text{m}$; width: $11.5-21.3-34.5\text{ }\mu\text{m}$ (Figs 42—43).

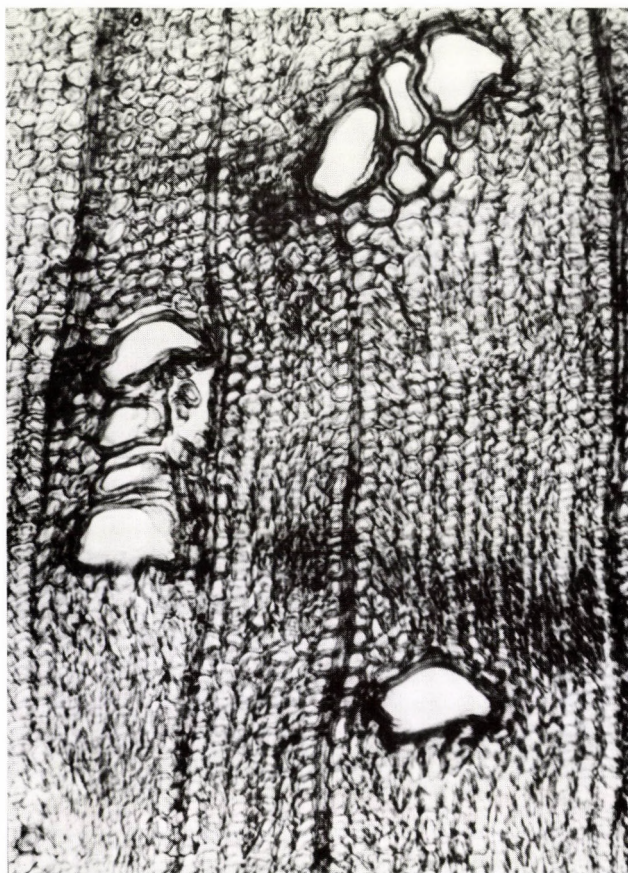


Fig. 41. Piptadenia flava (Spreng.)Benth. Cross-section 120 x. Vessels, medullary rays, fibres



Fig. 42. *Piptadenia flava* (Spreng.) Benth. Tangential section 120 x. One- and two-cell wide medullary rays. Vessels, longitudinal parenchyma and fibres. Septate crystal-holder longitudinal parenchyma (sp)

Fibre arranged in radial rows. Diameter: 11.6-16.7-20.9 μm ; wall thickness: 2.3-3.4-4.6 μm . Total length of fibres: 568.0-681.6-923.0 μm . Fibres end in short smooth tips. Septate crystal-holder longitudinal parenchyma with polygonal calcium oxalate crystals is not unfrequent in the xylem (Fig. 42).

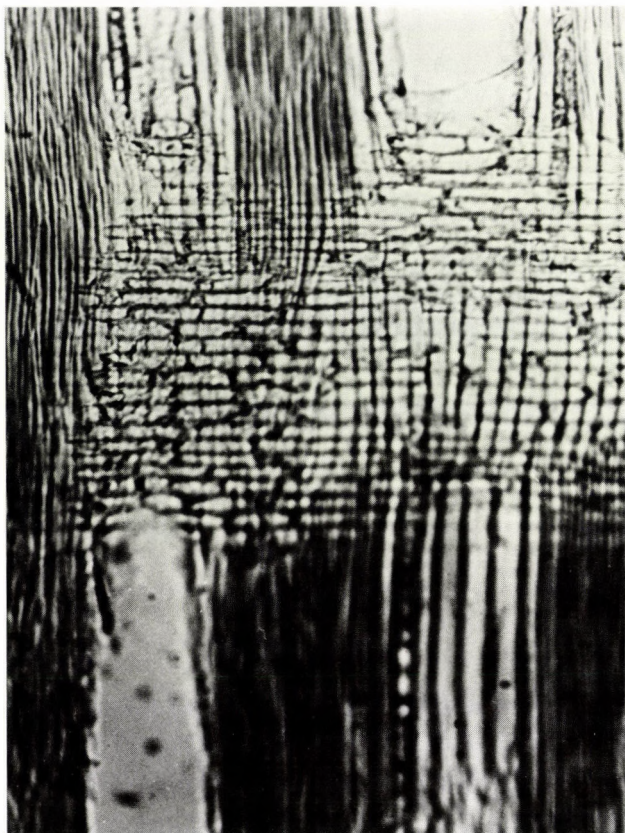


Fig. 43. Piptadenia flava (Spreng.) Benth. Radial longitudinal section 120 x. Heterogeneous medullary ray. Vessels, longitudinal parenchyma and fibres

REFERENCES

- Metcalfe, C. R., Chalk, L. (1950): Anatomy of the Dicotyledons. I. Clarendon Press, Oxford, 483—485.
- Sárkány, S., Szalai, I. (1964): Növény szerkezeti Gyakorlatok (Plant Anatomical Practicum). Tankönyvkiadó, Budapest, 559 pp.

ARCHITECTURE OF THE SHOOT SYSTEM OF
QUERCUS PETRAEA (MATT.)LIEBL.*

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The paper deals with the architecture of the shoot system of *Quercus petraea*. The examinations were carried out on the model area of the Síkfőkút Project and in its environment in 1986 and 1987. We found that the volume of the abscised oak shoots in the litter was $221.07 \text{ kg ha}^{-1} \text{ year}^{-1}$ and their number $814\,500 \text{ ha}^{-1} \text{ year}^{-1}$ on an average. The largest part of the abscised shoots are 1-2 years old. Abscission of shoots is continuous throughout the year reaching maximum in May and June. The Lepidoptera larvae consuming the leaves of the shoots greatly increase the number of abscised shoots, influencing thereby the spatial distribution and branching conditions of shoots remaining on the tree, even within a single period of vegetation, and affecting the pattern and efficiency of the photosynthesizing leaf mosaic.

The length of the shoots usually is 1-6 cm. The branching angles are above 50° on an average on the lower horizontal branches of elder trees, and between 30° and 65° on younger branches of orthotropous position. With a three years old regular forked shoot used as model, from the results of changing the angles of bifurcation it can be established that the leaf area most efficient from the point of view of light exploitation develops at branching angles of approximately 60° .

Keywords: Sessile oak, Síkfőkút Project, abscission, branching angle, leaf mosaic pattern

Introduction

The study of plant architecture on the basis of works by HORN (1971), LEOPOLD (1971) and HONDA (1971) became very intensive in the last two decades and is even now going on (cf. WATANABLE and KOIZUMI 1986; FITTER 1987; GELLER and NOBEL 1987; SAKAI 1987). It was then that the ecological importance of the architecture was realized; since then computer methods of simulation have been elaborated and are being used by an increasing number of authors (e.g. FISHER and HONDA 1979; HONDA et al. 1981; HUMPHREY and POWELL 1987).

*Síkfőkút Project No. 118.

The classification system widely used today, which describes 23 architecture models, was born from studies on the ontogeny of tropical trees in the first place (HALLÉ et al. 1978). On the basis of this system the development and structure of shoot system of Quercus petraea, an oak species dominant on the area of the Síkfőkút Project, can be described by means of the Rauh model. The architecture of plants building after the Rauh model is determined by the monopodial, rhythmically growing trunk and the multilevel branch system developed by it. The trees belonging here are polyaxial, all axes are orthotropous. The branches are morphogenetically identical with the trunk. The most important characteristic of the model is that the development of the branches is in close correlation with the rhythmical growth of the shoot axis. The branches of species in the temperate zone mainly develop proleptically. The plants are able to regenerate easily and quickly, since all meristems are equivalent and of rhythmical growth. The flowers always are lateral, and have no influence on the growth of the shoot system. Among the flowering plants this is the most frequent structural model.

After the Rauh model are built Hevea brasiliensis Muell.-Arg., a very important tree of the tropics; in the temperate zone e.g. Acer pseudo-platanus L., Calluna vulgaris Salisb. and Pinus silvestris L. These examples call attention to an essential factor in the course of applying the model. Although the building takes place in the same way, there are great differences between the four plant species mentioned and the sessile oak even in the shape, size and position of leaves. These differences made it reasonable to examine several structural elements of the shoot system of the sessile oak and their changes.

Method

The air-dry litter of 1986 and 1987 monthly collected from litter boxes of a total 10 m² ground area on the model area of the Síkfőkút Project was weighed, then the abscised sessile oak shoots contained in it were picked out. The shoots were classified by age (current year, year-old, two years old and older). On the basis of the data obtained the number and weight of abscised oak shoots per hectare, as well as the leaf area thus lost and the extent of modification of the shoot system were assessed.

On branches at different storeys of sessile oaks living on the model area and in its environment the average shoot lengths were measured to cm accuracy, while the branching angles with a 2-3° rounding off. The average leaf number of leafy shoots was determined. Several characteristic shoots and the leaf mosaic on them were analysed individually. These were used as model when by changing the angle of inclination of the branches the one most favourable for the maximum light exploitation of the leaf area was estimated.

Results and Discussion

When the investigations started the number of shoots of *Quercus petraea* on the model area of the Síkfőkút Project was 4 649 500 ha⁻¹, their weight 7929 kg ha⁻¹, and the leaf area 72 044 m² ha⁻¹ according to an estimate by JAKUCS and VIRÁGH (1975). With the individual number known (690 ind ha⁻¹), the average shoot number (6740 ind⁻¹ rounded off) and the leaf area of a shoot (155 cm²) can be calculated.

These average values are more or less modified both during a year and in the successive years, e.g. as a result of circannual growth rhythm, meteorological factors, defoliation, regeneration. It is therefore necessary to study the change of the shoot system in the course of analysing the architecture.

In the case of ligneous plants the abscission of various parts (leaves, flowers, fruits, shoots or even parts of root) can be regarded as a normal physiological process (KOZŁOWSKI 1973). In the course of the present work first the number, weight and age of the abscised shoots picked out from the litter were examined. The 1986 and 1987 data are included in Tables 1 and 2.

As seen from the tables the abscission of shoots while continuous throughout the year varies in intensity from season to season. Since the first sampling shows the joint result of the 4-month winter season it can be

Table 1

Number and weight of abscised sessile oak shoots in the litter on the model area of the Síkfőkút Project in 1986

Period	Total litter production kg ha ⁻¹	Shedded sessile oak shoots in litter					
		Weight kg ha ⁻¹	Current year pc ha ⁻¹	1-year old pc ha ⁻¹	2-year old pc ha ⁻¹	Older pc ha ⁻¹	Total pc ha ⁻¹
31. III.	486.67	38.00	0	47000	25000	37000	109000
30. IV.	131.04	25.32	3000	59000	26000	19000	107000
31. V.	393.62	65.98	19000	122000	35000	42000	218000
30. VI.	217.24	21.36	20000	32000	14000	24000	90000
31. VII.	232.92	14.43	19000	35000	13000	17000	84000
31. VIII.	234.12	7.31	22000	14000	4000	10000	50000
30. IX.	282.14	4.41	9000	2000	3000	6000	20000
31. X.	1921.25	15.72	29000	17000	12000	18000	76000
30. XI.	1521.07	6.13	13000	5000	3000	10000	31000
Total:	5420.07	198.66	134000	333000	135000	183000	785000

Table 2

Number and weight of abscised sessile oak shoots in the litter on the model area of the Sífőkút Project in 1987

Period	Total litter production kg ha ⁻¹	Shedded sessile oak shoots in litter					Total pc ha ⁻¹
		Weight kg ha ⁻¹	Current year pc ha ⁻¹	1-year old pc ha ⁻¹	2-year old pc ha ⁻¹	Older pc ha ⁻¹	
31. III.	884.20	74.82	0	64 000	23 000	4 000	91 000
30. IV.	147.23	32.22	0	57 000	21 000	16 000	94 000
31. V.	269.81	43.81	49 000	73 000	27 000	29 000	178 000
30. VI.	211.46	31.21	61 000	47 000	28 000	28 000	164 000
31. VII.	234.33	10.09	22 000	14 000	11 000	8 000	55 000
31. VIII.	171.85	9.25	45 000	18 000	12 000	10 000	85 000
30. IX.	531.78	11.46	42 000	17 000	9 000	4 000	72 000
31. X.	1238.63	17.65	21 000	8 000	5 000	18 000	52 000
30. XI.	1459.21	12.97	16 000	11 000	13 000	13 000	53 000
Total:	5148.50	243.48	256 000	309 000	149 000	130 000	844 000

established that the number of abscised shoots is on an average the lowest in this period (25 000 number ha⁻¹ a month). In the course of April, May and June intensive increase can be observed. The number and weight of abscised shoots reached maximum in May both years.

Remarkable is the age distribution of shoots. The proportion of shoots older than two years is relatively low, only 19 per cent on the average of the 2 years concerned. The abscised shoots are mostly 1-2 years old, or formed and withered in the current year. The proportion of the total branch-litter production fits well into the data of litter production measuring having taken place on the model area since 1973 (TÓTH *et al.* 1985). Since owing to the destruction of oaks the number of standing sessile oaks was reduced on the Project to 310 ind ha⁻¹ by the end of 1987 (JAKUCS *ex verb.*), from one oak-tree abscission of an average of 2627 number year⁻¹ shoots can be reckoned with. According to the data of JAKUCS (1985) the surface of a sessile oak leaf on a 5-year average is 21.08 cm². With this value taken into consideration, the number of shoots abscised a year means the loss of a round 1 ha⁻¹ leaf area in the period concerned. It should be noted that phytophages caused considerable defoliation both years.

From earlier investigations it is known that as a result of a serious defoliation taking place early in spring a considerable proportion of the

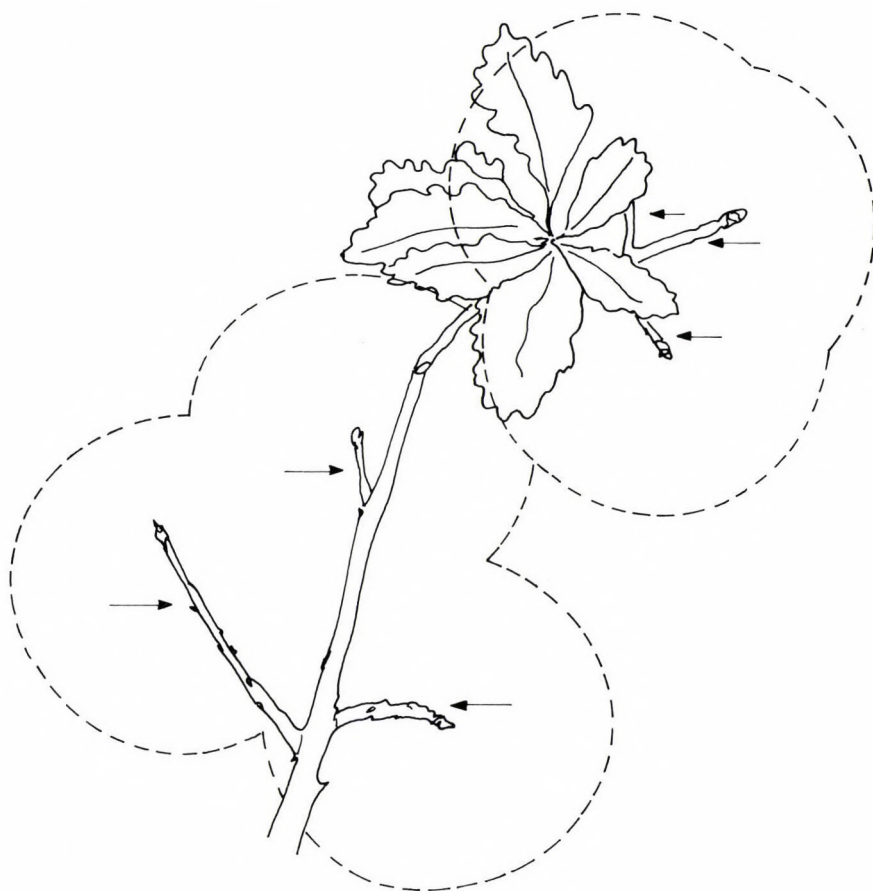


Fig. 1. Changes in the leaf mosaic within a vegetation period in consequence of feeding by Lepidoptera larvae. The arrows show the shoots chewed and abscised, the arcs the original leaf mosaic. The six shoots destroyed were replaced by one

current year shoots may wither and get into the litter (NAGY 1981). This explains the abscission maximum observed in May and the high June values. A part of the shoots abscised when one-year old did not lignify in the previous year due to late development or powdery mildew infection, and were destroyed in the severe cold — these fall off mostly in the first half of the year. In another part of the young shoots the abscission tissue develops for other reasons (e.g. intensive self-shading).

In consequence of defoliation regeneration shoots often develop in masses in the oak, or in the case of a lower defoliation stress proleptic

Table 3

Average length of one-year shoots in 25 trees

Length of shoots, cm	Rate, % composed to total shoots
1-3	62
4-6	25
7-12	10
13- <	3

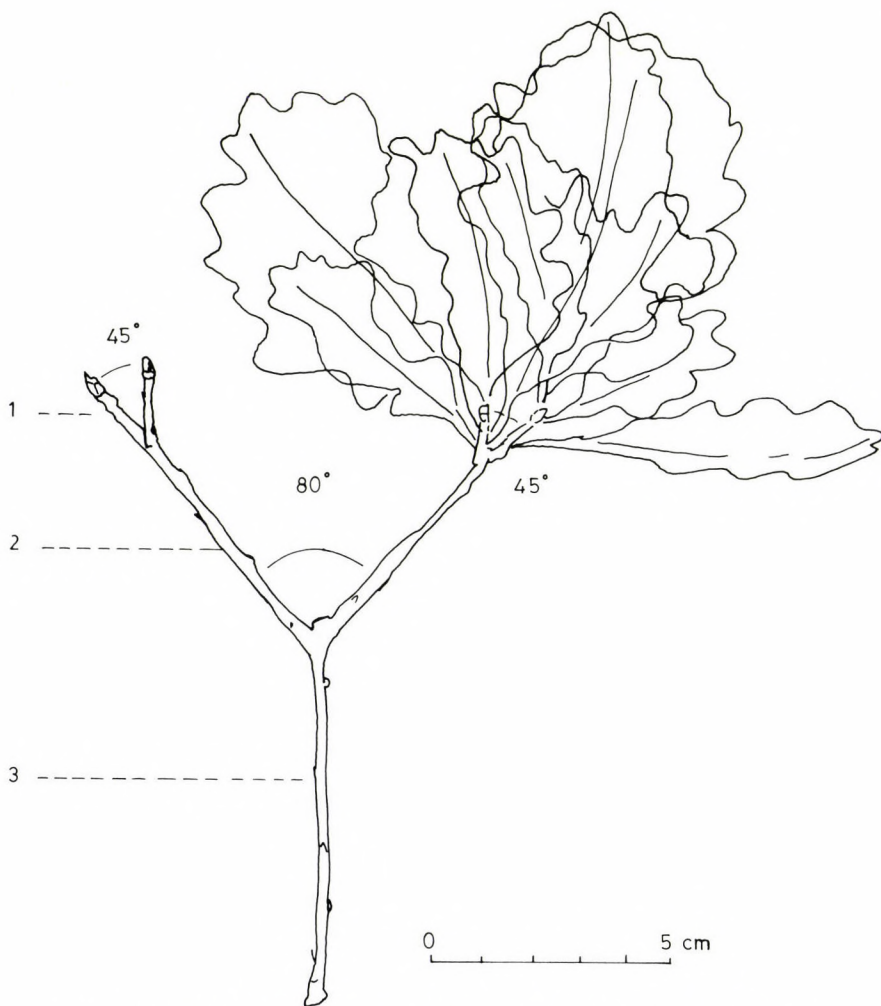


Fig. 2. Twig of regular bifurcation with the leaf mosaic of two shoots.

1, 2, 3: one-, two- and three-year shoot; 45°: angle of bifurcation

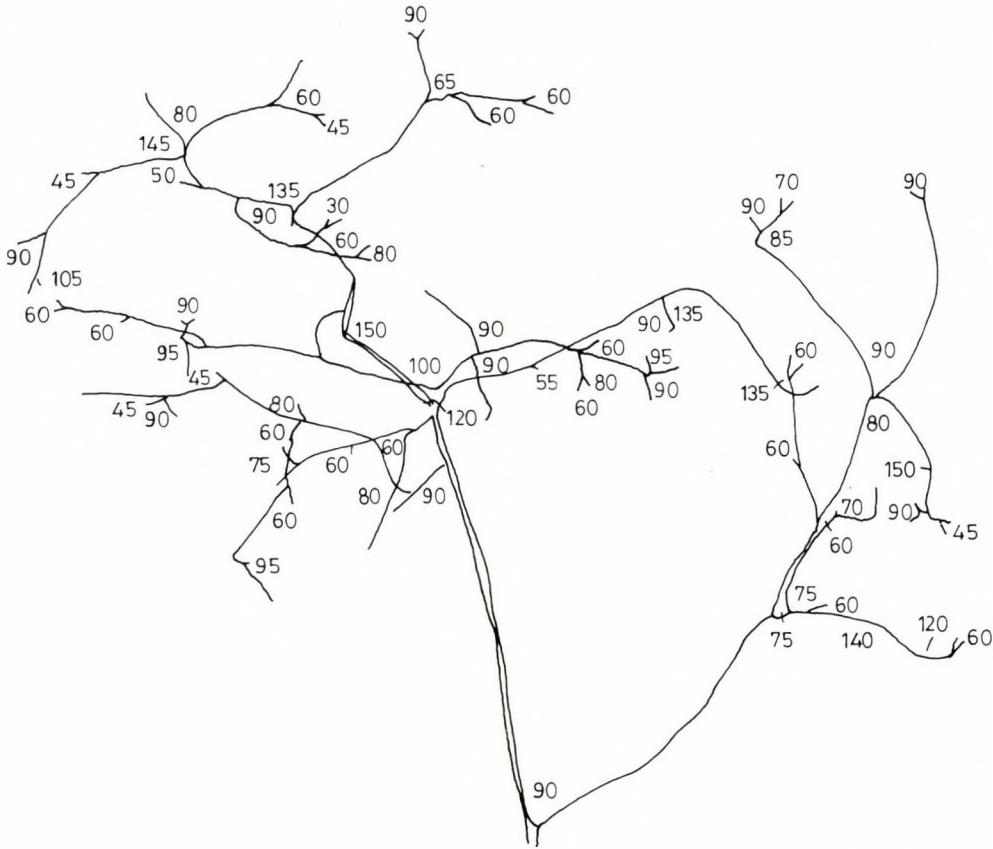


Fig. 3. Branching and inclination angles on a horizontal lower branch of an old oak

shoots characteristic of oaks. As a result, the number, position, branching angle of shoots bearing the photosynthesizing leaf surface, and the size and shading effect of the leaf mosaic on them are greatly modified even within one vegetation period. Figure 1 shows the initial shoots and reconstructed leaf mosaic of a totally defoliated twig, and the regenerated, still growing leaf area, well illustrating an extreme example of changes taking place during the year.

The length of shoots was examined on the lateral branches of 20 old trees chosen at random, and on orthotropous branches of 5 younger offsets (Table 3). According to the table 87 per cent of the one-year shoots are 1-6 cm long, and only 3 per cent of them exceeds 12 cm. Longer shoots are characteristic at the orthotropous branch tips of younger trees.

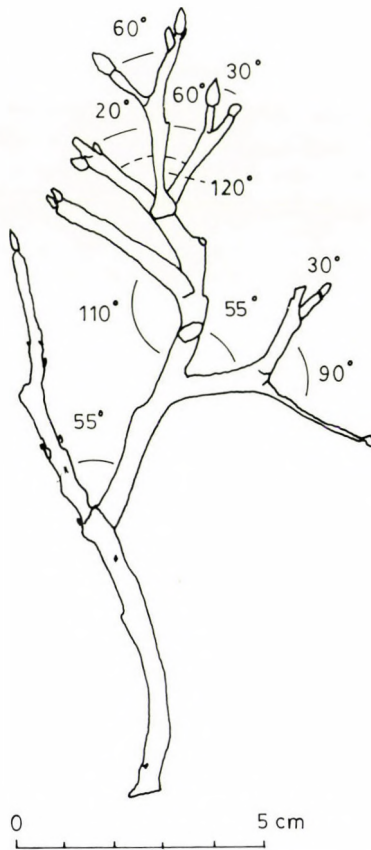


Fig. 4. Characteristic inclinations and shoot pattern on the lateral branch end of an old oak

The number of leaves on shorter shoots is 4-9 (Fig. 2), on longer ones 12-20. The divergence angle of the leaves is 144° , that is, the phyllotaxis is $2/5$.

By computer simulation of the branch system of Terminalia catappa L., a tree with very regular architecture, HONDA and FISHER (1978) have pointed out that the inclination of the bifurcations of branches determines the leaf area. They found that in Terminalia trees possessing primary, secondary and tertiary branches the theoretically possible maximum leaf area developed at 66° angles of inclination. The actually measured bifurcation angles were about 61° .

Table 4

Branching angles on branches of young and old oaks

Branching angles	Rate %, composed to total shoots	
	On young oaks	On old oaks
30—45°	60	6
50—65°	36	42
70—<°	4	52

The branch- and shoot system of oaks is far from being as regular as those of *Terminalia* or of the similarly tropical *Tabebuia rosea* DC. (BORCHERT and TOMLINSON 1984). Particularly the horizontal lower oak branches curve and bend in all directions (Fig. 3) though the bifurcation and branching angles of younger shoots also show high diversity (Fig. 4).

As seen from Table 4 the frequency of the branching angles is rather different in the elder trees examined compared to the younger trees. On the orthotropous branches of the apical part of young trees the proportion of branching between 30 and 45° is much larger, bifurcate end-branching is fewer, and then the angle of bifurcation usually is above 40°. On horizontal branches of older trees it is the other way round.

The three years old twig of regular bifurcation shown in Fig. 2 was used as a model to estimate the correlations between the effective leaf area and the angle of inclination of shoots. With the twig proportionately reduced in size, the inclinations of the primary and secondary shoots were changed, the idealized leaf area (marked with a circle) was left unchanged (Fig. 5).

Considering the thousands of shoots present on several storeys of a tree, on the basis of the figures it can be established that in the case of a bifurcate branch system an angle of inclination about 60° ensures the maximum effective leaf area, that is the least covering beside the best exploitation of space, for the oak too. We have seen in Table 4 that a large proportion of the angles measured falls within this range, while others range between wide limits.

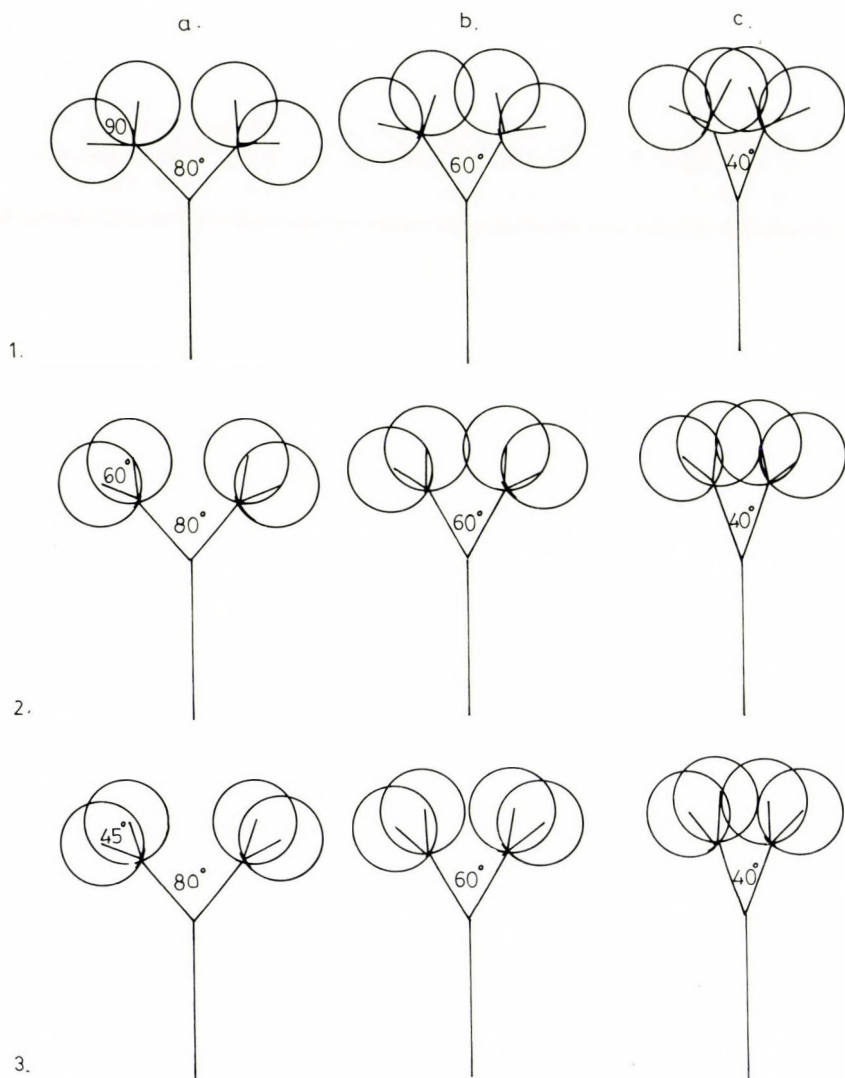


Fig. 5. Changes in the effective leaf area at different angles of inclination. Inclinations of one-year branches: 1 = 90° , 2 = 60° , 3 = 45° . In part 3.a of this figure the model of the branch shown in Fig. 2 can be seen

REFERENCES

- Borchert, R., Tomlinson, P. B. (1984): Architecture and crown geometry in Tabebuia rosea (Bignoniaceae). Amer. J. Bot. **71**: 958—969.
- Fisher, J. B., Honda, H. (1979): Branch geometry and effective leaf area: a study of Terminalia-branching pattern. 1. Theoretical trees. Amer. J. Bot. **66**: 633—644.
- Fitter, A. H. (1987): An architectural approach to the comparative ecology of plant root systems. New Phytol. **106** (Suppl.): 61—77.
- Geller, G. N., Nobel, P. S. (1987): Comparative Cactus architecture and PAR interception. Amer. J. Bot. **74**: 998—1005.
- Hallé, F., Oldeman, R. A. A., Tomlinson, P. B. (1978): Tropical trees and forests. An architectural analysis. Springer-Verl., Berlin, Heidelberg, N. Y.
- Honda, H. (1971): Description of the form of trees by the parameters of the tree-like body: effects of the branching angle and the branch length on the shape of tree-like body. J. Theor. Biol. **31**: 331—338.
- Honda, H., Fisher, J. B. (1978): Tree branch angle: maximizing effective leaf area. Science **199**: 888—890.
- Honda, H., Tomlinson, P. B., Fisher, J. B. (1981): Computer simulation of branch interaction and regulation by unequal flow rates in botanical trees. Amer. J. Bot. **68**: 569—585.
- Horn, H. S. (1971): The adaptive geometry of trees. Princetown Univ. Press, Princetown—New Jersey.
- Jakucs, P. (1985): Structure of the forest. In: Jakucs, P. (ed.): Ecology of an oak forest in Hungary. Results of "Síkfőkút Project". Akad. Kiadó, Budapest, pp. 107—126.
- Jakucs, P., Virágh, K. (1975): Changes in the area and weight of light- and shade-adapted leaves and shoots of Quercus petraea and Quercus cerris in a Hungarian oak forest ecosystem. Acta Bot. Acad. Sci. Hung. **21**: 25—36.
- Kozlowski, T. T. (1973): Shedding of plant parts. Acad. Press, N. Y., London.
- Leopold, L. B. (1971): Trees and streams: the efficiency of branching patterns. J. Theor. Biol. **31**: 339—354.
- Nagy, M. (1981): The effect of Lepidoptera larvae consumption on the leaf production of Quercus petraea (Matt.) Liebl. Acta Bot. Acad. Sci. Hung. **27**: 141—150.
- Remphrey, W. R., Powell, G. R. (1987): Crown architecture of Larix laricina samplings: an analysis of higher order branching. Can. J. Bot. **65**: 268—279.
- Sakai, S. (1987): Pattern of branching and extension growth of vigorous samplings of Japanese Acer species in relation to their regeneration strategies. Can. J. Bot. **65**: 1578—1585.
- Tóth, J. A., Papp, L. B., Jakucs, P. (1985): Litter production of the forest. In: Jakucs, P. (ed.): Ecology of an oak forest in Hungary. Results of "Síkfőkút Project". Akad. Kiadó, Budapest.
- Watanable, M., Koizumi, H. (1986): Correlation between trunk and branch age in trees as a new parameter in the analysis of tree architecture. Vegetatio **68**: 103—108.

CYTOLOGICAL STUDY OF ANTHOCYANIN PRODUCTION IN GRAPEVINE (VITIS VINIFERA L.) CALLUS CULTURES

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Anthocyanin producing calli were established from shoot explants of three clones of a red vine hybrid 'Castets' x 'Abouriou noir' (*Vitis vinifera* L.) after placing them on MS' medium with Gamborg's vitamins, 30 g.l⁻¹ sucrose and various combination of growth regulators. Anthocyanin producing cells were located at peripheries of cell clumps from which very friable calli were composed of. These cells contained cytoplasm rich in cell organelles including amyloplasts and abundant profiles of endoplasmic reticulum. The presence of osmiophilic material forming globuli, irregular bodies or layers in differently large vesicles located in the cytoplasm and layers, globuli, and precipitations in central vacuoles was a characteristic feature of these cells. Alongside the osmiophilic material unusual balls of channels having dipped into the central vacuoles or larger cytoplasmic vesicles can be seen. The vacuole globuli as red coloured objects (anthocyanoplasts) varying size and number were visible in a light microscope. No osmiophilic material and balls were present in cells of young calli or calli of nonproducing variety 'Riesling'.

Introduction

Anthocyanins, red to blue coloured flavonoids, are widely spread among plants. Much attention has recently been paid to regulation and enzymology of anthocyanin production (COE et al. 1988; VAN DER KROL et al. 1990). Although the biochemical pathway of anthocyanin synthesis is more or less known, the precise localisation of successive steps in the cells has not been completely understood. A suitable model for studying these aspects might be cells cultured in vitro. It is well known that callus and cell cultures of many genera including grapevine (YAMAKAWA et al. 1983a, b; TAMURA et al. 1989; DO and CORMIER 1990) are capable of strong anthocyanin

Abbreviations: cw = cell wall, er = endoplasmic reticulum, d = dictyosome, m = mitochondria, n = nucleus, nu = nucleolus, v = vacuole, BAP = 6-benzyl-aminopurine, IBA = indole-3-butyric acid, 2,4-D = 2,4-dichlorophenoxyacetic acid.

production. This paper presents results of cytological investigations of anthocyanin synthesis in calli of a pigmented grapevine hybrid.

Material and Methods

Pigmented calli were established from young sprouts of three clones of a grapevine (*Vitis vinifera* L.) hybrid 'Castets' x 'Abouriou noir'. The sprouts buded from woody springs, collected on the field in winter time, after putting them to water. Explants about 3 mm long were placed into 100 ml Erlenmeyer flasks with 30 ml medium containing MS' salts (MURASHIGE and SKOOG 1962), Gamborg's vitamins (GAMBORG *et al.* 1968), $30 \text{ g} \cdot \text{l}^{-1}$ sucrose, $0.05 \text{ mg} \cdot \text{l}^{-1}$ kinetin and $0.5 \text{ mg} \cdot \text{l}^{-1}$ 2,4-D, resp. $0.226 \text{ mg} \cdot \text{l}^{-1}$ BAP, $0.03 \text{ mg} \cdot \text{l}^{-1}$ IBA, $80 \text{ mg} \cdot \text{l}^{-1}$ adenine, $170 \text{ mg} \cdot \text{l}^{-1}$ NaH_2PO_4 . After adjustment of pH at 5.2 the medium was solidified with $7 \text{ g} \cdot \text{l}^{-1}$ agar. The explants were kept under permanent illumination of 3000 lx and at a temperature of 25 °C.

Samples for cytological studies were taken from calli after 14 and 28 days, fixed with 3% glutaraldehyde for 3 h, postfixed with 2% osmium tetroxide for 2 h, dehydrated in acetone and embedded in Durcupan ACM Fluka. Semithin sections were prepared and stained by basic fuchsin and toluidine blue (LUX 1981), thin sections by uranylacetate and lead citrate (REYNOLDS 1963) and investigated in Tesla BS 613 or JEM 2000 FX microscopes. Anthocyanins were extracted with methanol with 1% HCl from fresh calli overnight at 4 °C and absorbance of solution was measured. Calli from sprouts of anthocyanin-nonproducing variety 'Riesling' were also established and used for comparison.

Results and Discussion

Calli of all clones and variety 'Riesling' were white or pale green at first and contained highly vacuolised cells (Fig. 1a, b). The cytoplasm of nondividing cells was reduced to thin layers at the margins of the cells. Organellae including nuclei were placed along the cell walls. Phragmosomes and cytoplasmic strands were formed during prophase in highly vacuolised dividing cells. The pattern of division of these vacuolised cells was similar as described earlier (JÁSIK and HUDÁK 1989).

After 28 days of cultivation the callus cultures of the hybrid plants were friable and turned red. The pigments exhibit maximal absorbance at 530 nm. On nonfixed samples of squashed calli red coloured and colourless cells were observed. Some of cells contained strongly pigmented globuli which were varying in numbers and sizes. Such globuli termed anthocyano-plasts have been observed by other authors studying anthocyanin producing cells cultured in vitro (NOZUE and YASUDA 1985; NOZZOLILLO and ISHIKURA 1988). Semithin sections showed inner organization of cell clumps in the friable calli (Fig. 1c). Smaller, somewhat more cytoplasmic cells, with plastids packed with starch grains, located mainly around spherical nuclei, were present in the central parts of cell clumps (Fig. 1c). No dense deposits were observed in these cells. Electronmicroscopical study confirmed

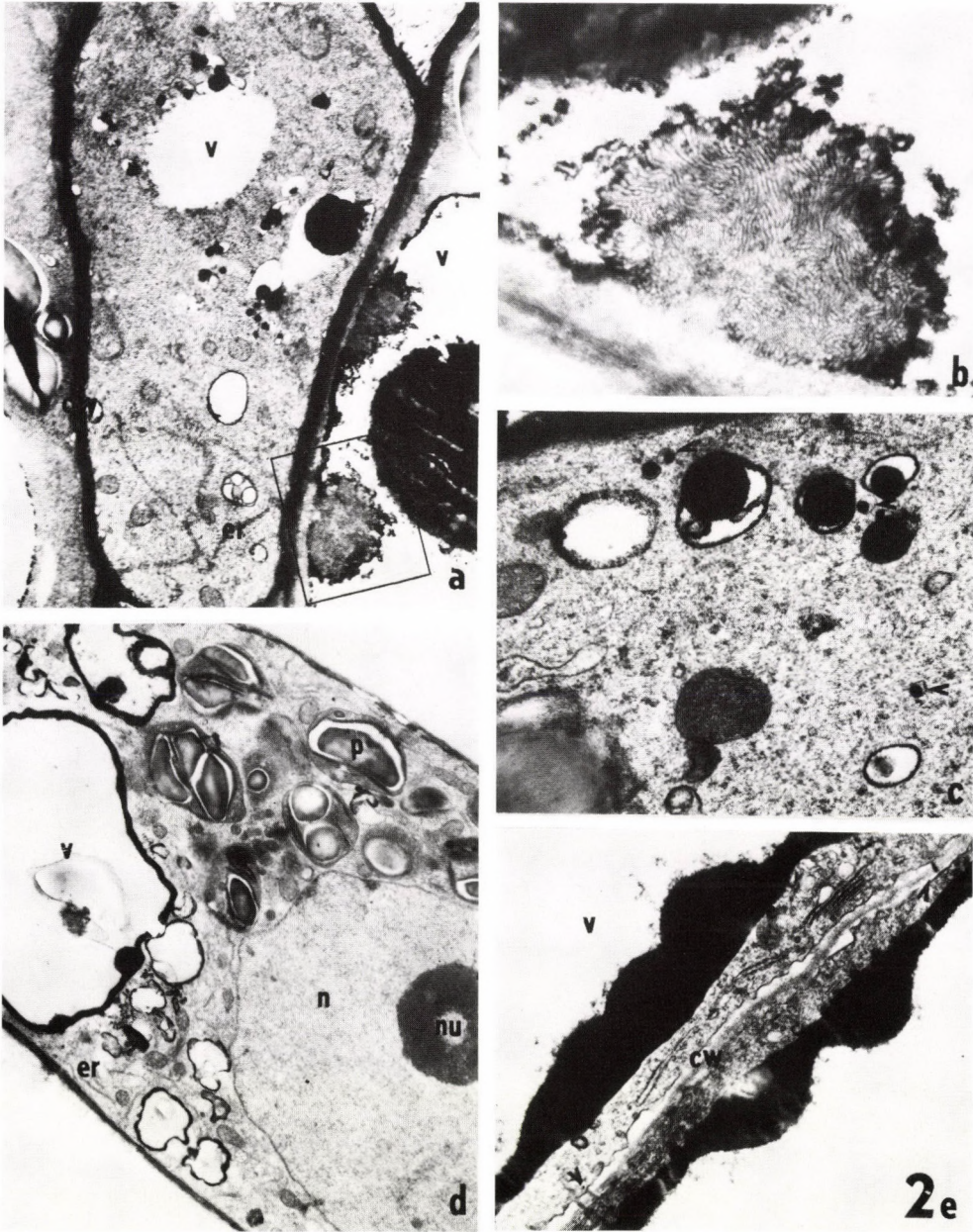


Fig. 2. a) The cytoplasm of the anthocyanin producing cell with numerous vesicles contained osmiophilic deposits (the enclosed area is shown at higher magnification in Fig. 2 b), 7400 x; b) The ball of channels dipping into the central vacuole, 23000 x; c) The portion of cytoplasm with numerous ribosomes and vesicles. The smallest osmiophilic globuli (arrowheads) are encircled by a membrane as well, 33 200 x; d) Cytoplasm with nucleus surrounded by cell organelles, 5500 x; e) The layers of osmiophilic material on the inner sides of the tonoplasts of anthocyanin producing cells. There are plasmodesmata connections (arrowheads) between such cells, 21 000 x

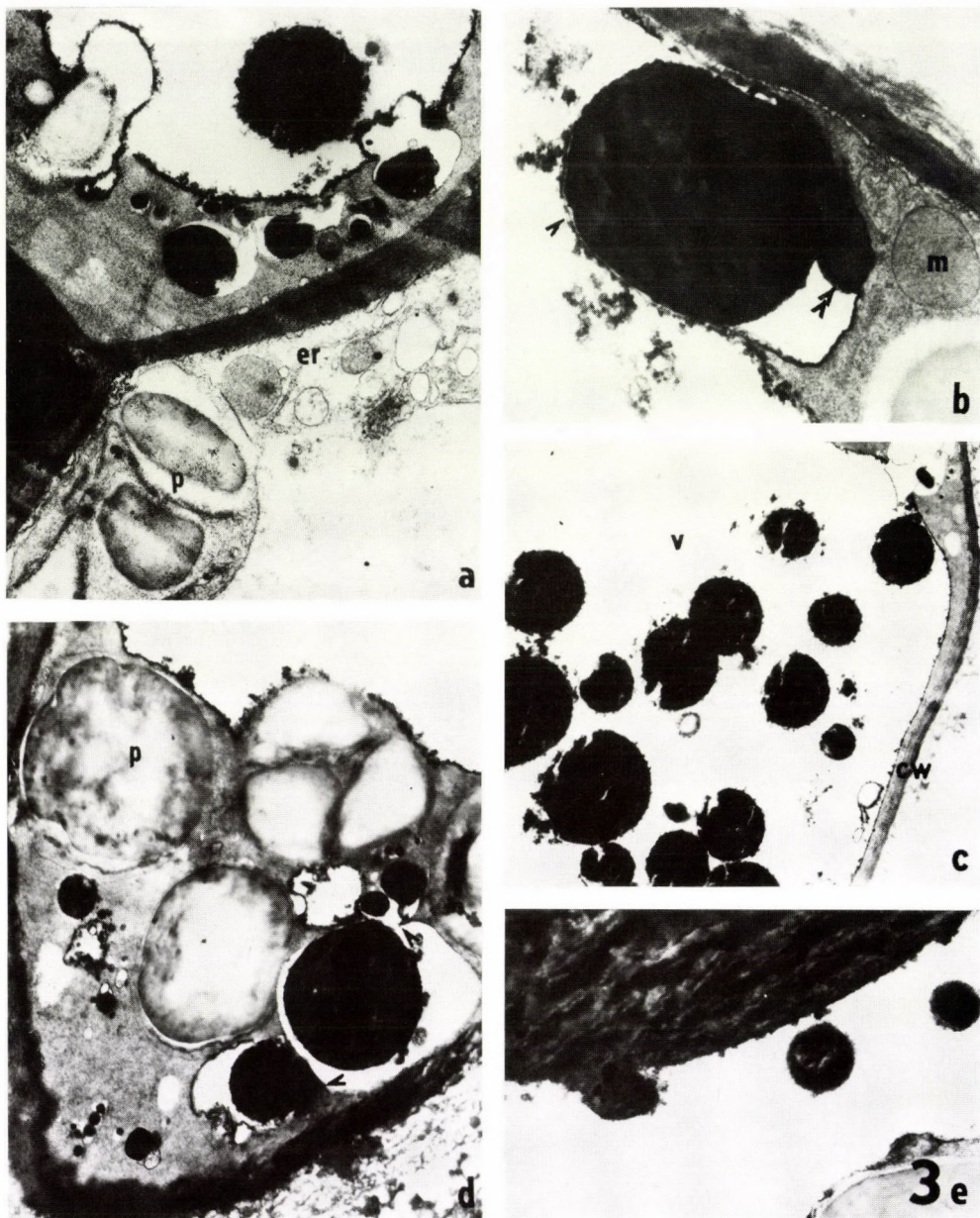


Fig. 3. a) The thin section through the parts of anthocyanin producing and nonproducing cells. The osmiophilic material in different forms can be seen in the first one, 11500 x; b) A large globula near the ball of channels (double arrowhead) being loosen into the central vacuole. The tonoplast attached to the membrane of the vesicle is visible (arrowhead) on its surface, 28 800x; c) Osmiophilic globuli (anthocyanoplasts) of varying sizes located in the central vacuole, 6000 x; d) A portion of cytoplasm with vesicles contained osmiophilic material. Some of them fuse together (arrowheads), 16 900 x

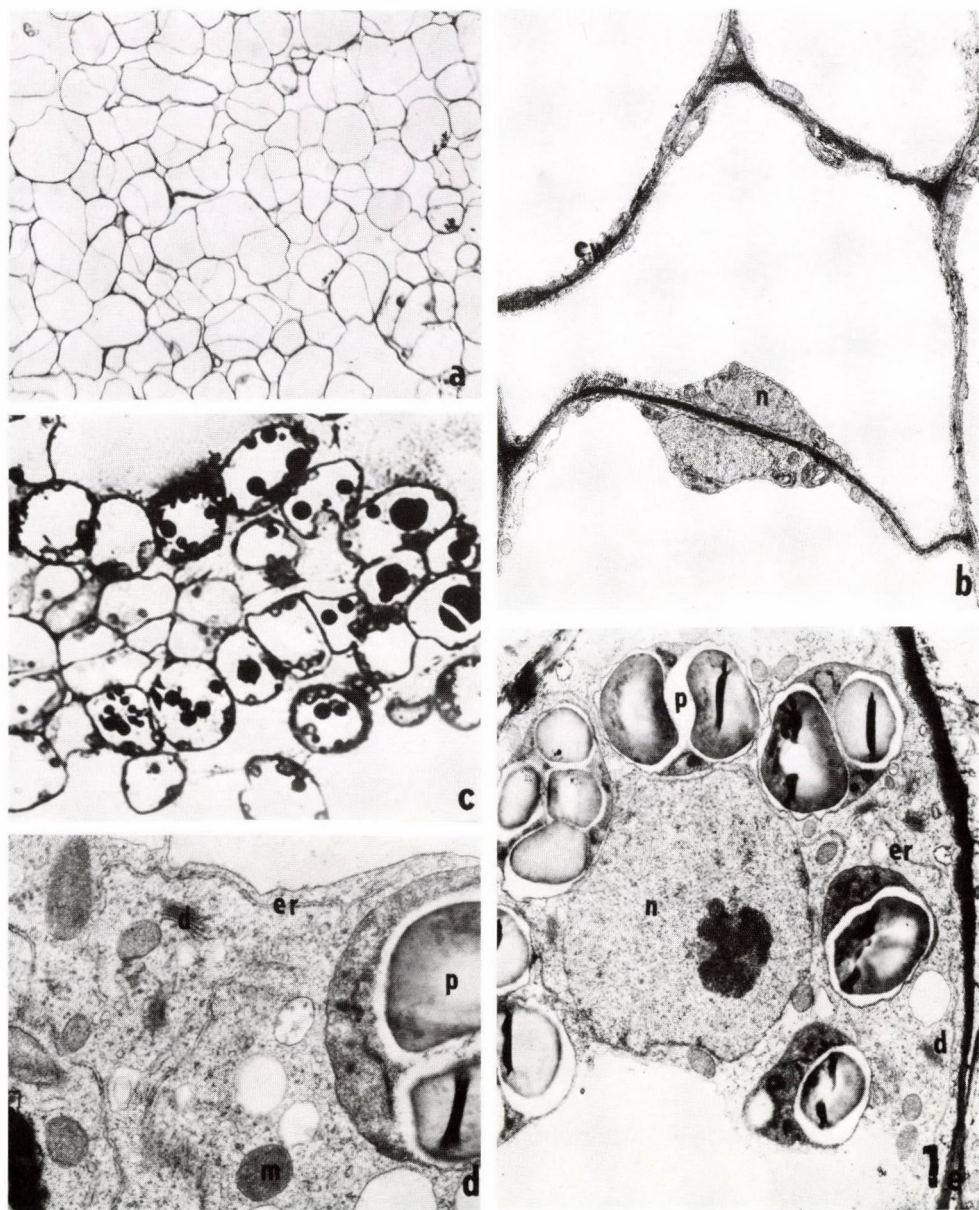


Fig. 1. a) The semithin section through the callus of the hybrid 'Castets' x 'Abouriou noir' with highly vacuolised, nondividing cells (14 days after placement) 190 x; b) The vacuolised cell in the calli of the variety Riesling (after 28 days of cultivation) 3100 x; c) The semithin section through the cell clump of the friable callus of the hybrid 'Castets' x 'Abouriou noir' 310 x; d) The cytoplasm rich in cell structures of the cell located in the central part of the cell clump 18600 x; e) The nucleus encircled with amyloplasts in the cell of the central zone of the cell clump 7800 x

the presence of large starch grains. Nuclei contained small nucleoli together with slightly dispersed chromatin (Fig. 1e). The cytoplasm was rich in cell structures (Fig. 1e, d). Many vesicles were derived from margins of cisternae of endoplasmic reticulum and dictyosomes (Fig. 1d). Osmiophilic material neither in cytoplasm nor in vacuoles was present. At the peripheries of the cell clumps larger cells with starch grains and nucleus in the cytoplasm and one large or several smaller dark globuli in vacuoles (visible even without staining) were seen on semithin sections (Fig. 1c). The cytoplasm rich in cell structures was noticed on ultrathin sections. In cell walls there were plasmodesmata (Fig. 2e). Such connections among cells cultured in vitro are usually absent but in the case of relatively specialised cells the interconnections by plasmodesmata for their coordination are perhaps necessary. On some sections unusual balls of tubules (channels) were found (Fig. 2a, b). These complexes were localised in large vesicles situated in cytoplasm (Fig. 3b) as well as at peripheries of central vacuoles (Fig. 2a, b). Osmiophilic material in these cells formed globuli (Figs 2a, c, 3a—d) irregular bodies, thin layers (Fig. 2c, d) being present inside of the vesicles located in the cytoplasm. These vesicles varying in size often fused together (Fig. 3d) and with central vacuoles (Fig. 3a, b). Similar osmiophilic material was present on the inner faces of tonoplasts (Figs 2d, e, 3a). Dense precipitations were found out near these layers (Figs 2e, 3a) as well as the balls mentioned above (Fig. 2b). Some osmiophilic globuli of various number and size were scattered in the central vacuoles (Fig. 3a, c). On many sections some precipitations and smaller globuli were attached on surfaces of larger globuli (Fig. 3a, e). No such osmiophilic material was present in the calli of the variety 'Riesling'. Therefore, it is supposed that osmiophilic material represent some intermetabolites of the anthocyanin pathway or their insoluble complexes with some other compounds. However, the first steps of synthesis are carried out somewhere in the cytoplasm. It is well documented that the key enzymes of this synthesis are dissolved in cytoplasm, or they are associated with the cytoplasmic face of endoplasmic reticulum cisternae and perhaps with the tonoplast (FRITSCH and GRISEBACH 1975; HRAZDINA *et al.* 1987; BEERHUES and WIERMANN 1988). The osmiophilic material was never placed freely in the cytoplasm but it was located in vesicles. Intermetabolites had to traverse the membrane of the vesicles and become osmiophilic. The vesicles might be derived from endoplasmic reticulum profiles. By fusion of these vesicles with the central vacuole,

dense material is loosened into it. The same way of transport was postulated by PARHAM and KAUSTINEN (1977) for the similar compound of tannins. On the other hand, the osmiophilic material was present on the inner face of tonoplast in the form of layers. Intermetabolites could also permeate directly into the central vacuoles across tonoplasts. This is in good agreement with the suggestion of HOPP and SEITZ (1987) that anthocyanin synthesis also takes place on cytosolic face of the tonoplast. The mechanism of traversing the tonoplast and the vesicle membrane might be similar. Nevertheless, the osmiophilic deposits in vesicles or the central vacuole may represent considerably advanced intermetabolite of flavonoid pathway, perhaps acylated by now. According to HOPP and SEITZ (1987) acylation in the case of anthocyanins plays a fundamental role in their transport and storage in vacuoles. NAKAMAE and NAKAMURA (1983) also found that the transport into the vacuole is mediated by tonoplast ATPase and can be stimulated by UV light.

A well-known feature of anthocyanin producing cells seems to be the presence of red globuli termed anthocyanoplasts (SMALL and PECKET 1982). These structures were observed also in cultured cells (NOZUE and YASUDA 1985; NOZZOLILLO and ISHIKURA 1988). Although NOZZOLILLO and ISHIKURA (1988) suggested that red globuli may be localised in cytoplasm as well, pigmented material forming globuli varying in size and number generally is placed in central vacuoles. Coloured subjects found in the cytoplasm by the authors mentioned above can be identical with larger osmiophilic globuli located in the cytoplasmic vesicles observed in the cells of the grapevine calli by the electron microscope as well. Although a precise role of the coloured globuli has not been fully cleared up there are some suggestions that they take part in modification, perhaps in accumulation of anthocyanins (SMALL and PECKET 1982; NOZUE and YASUDA 1985). In the case of the grapevine calli, the coloured globuli appeared at the specific ontogenetic stage of the cells, when the calli were turning red. They were evidently formed from osmiophilic precipitations loosened from dense layers placed on the inner faces of tonoplasts. The globuli might be a place of conversion of osmiophilic substance into soluble pigments staining whole vacuolar content.

Results of electronmicroscopical investigations of the anthocyanoplasts are controversial. According to SMALL and PECKET (1982) there is a membrane barrier around the globuli located in vacuoles. At the same time in some cases, enclosures exhibited certain inner structural organization. On the other hand NEUMANN (1983) failed to find the membrane and inner arrangement and account them for hydrophobic droplets. Our observations

confirm later point of view. An additional membrane has never been observed in the case of grapevine callus cells. Eventual membrane covering portion of globuli, visible on some sections (Fig. 3b) represent always the tonoplast or the membrane of cytoplasmic vesicle. Designation of droplets, not surrounded by membrane and without inner organization as anthocyanoplasts is not quite correct then as this term strongly suggests an organelle character of them.

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REFERENCES

- Beerhues, L., Wiermann, R. (1988): Chalcone synthases from spinach (*Spinacea oleracea* L.). I. Purification, peptide patterns, and immunological properties of different forms. Planta **173**: 532–543.
- Coe, E. H., Neuffer, M. G., Hoisington, D. A. (1988): The genetics of corn. In: Sprague, G. F., Dudley, J. K. (eds): Corn and Corn Improvement. Amer. Soc. Agron., pp. 81–258.
- Do, C. B., Cormier, F. (1990): Accumulation of anthocyanins enhanced by high osmotic potential in grape (*Vitis vinifera* L.) cell suspensions. Plant Cell Reports **9**: 143–146.
- Fritsch, H., Grisebach, H. (1975): Biosynthesis of cyanidin in cell cultures of *Haplopappus gracilis*. Phytochemistry **14**: 2437–2442.
- Gamborg, O. L., Miller, R. A., Ojima, K. (1968): Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. **50**: 151–158.
- Hopp, W., Seitz, H. U. (1987): The uptake of acylated anthocyanin into isolated vacuoles from a cell suspension culture of *Daucus carota*. Planta **170**: 74–85.
- Hrazdina, G., Zobel, A. M., Hoch, H. C. (1988): Biochemical, immunological and immunocytochemical evidence for the association of chalcone synthase with endoplasmic reticulum membranes. Proc. Nat. Acad. Sci. USA **84**: 8966–8970.
- Jásik, J., Hudák, J. (1989): The electronmicroscopical study of the division of highly vacuolised grapevine callus cells. Biol. Plant. **31**: 321–326.
- Lux, A. (1981): Rýchla metóda farbenia polotenkých rezov z rastlinných objektov. Biológia **36**: 753–757.
- Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. **15**: 473–497.
- Nakamae, H., Nakamura, N. (1983): Effects of metabolic inhibitors on anthocyanin accumulation in petals of *Rosa hybrida* Hort. cv. Ehigasa. Plant Cell Physiol. **24**: 995–1002.
- Neumann, D. (1983): Subcellular localization of anthocyanins in red cabbage seedlings. Biochem. Physiol. Pflanzen **178**: 405–407.
- Nozue, M., Yasuda, H. (1985): Occurrence of anthocyanoplasts in cell suspension cultures of sweet potato. Plant Cell Reports **4**: 252–255.

- Nozzolillo, C., Ishikura, N. (1988): An investigation of the intracellular site of anthocyanoplasts using isolated protoplasts and vacuoles. Plant Cell Reports 7: 389—392.
- Parham, R. A., Kaustinen, H. M. (1977): On the site of tannin synthesis in plant cells. Bot. Gaz. 138: 465—467.
- Reynolds, S. (1963): The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17: 208—212.
- Small, C. J., Pecket, R. C. (1982): The ultrastructure of anthocyanoplasts in red-cabbage. Planta 154: 97—99.
- Tamura, H., Kumaoka, Y., Sugisawa, H. (1989): Identification and quantitative variation of anthocyanins produced by cultured callus tissue of Vitis sp. Agric. Biol. Chem. 53: 1969—1970.
- Van der Krol, A. R., Mur, L. A., Beld, M., Mol, J. N. M., Stuitje, A. R. (1990): Flavonoid genes in petunia: Addition of a limited number of gene copies may lead to a suppression of gene expression. Plant Cell 2: 291—299.
- Yamakawa, T., Ishida, K., Kato, S., Kodama, T., Minoda, Y. (1983a): Formation and identification of anthocyanins in cultured cells of Vitis sp. Agric Biol. Chem. 47: 997—1001.
- Yamakawa, T., Kato, S., Ishida, K., Kodama, T., Minoda, Y. (1983b): Production of anthocyanins by Vitis cells in suspension culture. Agric. Biol. Chem. 47: 2185—2191.

HISTOMORPHOLOGICAL STUDIES OF TRACHEARY ELEMENTS AND CHEMICAL COMPOSITION OF *Khaya ivorensis* A. JUSS (MELIACEAE)

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Investigations were carried out on the variation pattern in the dimensions of fibre and vessel traits along both vertical and horizontal axes of the plant. Variation in the percentage of cellulose, lignin and hemicellulose contents along the vertical axis was also investigated. Histomorphological parameters viz: fibre length (F_l), fibre diameter (F_d), Lumen diameter (L_d), fibre wall thickness (W_t), Runkel ratio (R_r), Flexibility coefficient (F_c), Relative fibre length (R_{fl}), Vessel length (V_l), Vessel diameter (V_d) and Fibre/Vessel length ratio (F/V) were determined. Mean F_l ranged from 1120.6–1500.06 µm, F_d 17.14–23.83 µm, R_f 154.94–74.59, V_l 326.9–536.2 µm, V_d 51.83–162.42 µm, and F/V 2.55–3.55–3.78. Plant height had significant effect on vessel length. The distance from the pith outwards had no significant on all the parameter studied. Plant height had no significant effect on the percentage composition of cellulose, lignin and hemicellulose and the mean values for these components were 38.5%, 25.5% and 36.75%, respectively.

Introduction

Khaya A. Juss (Meliaceae), a tropical African genus of 8 aborescent species (WILLIS and AIRY-SHAW 1973) and from Nigeria four species have been reported (KEAY *et al.* 1964). The earlier contributors on the histomorphology of the genus *Khaya* are GILL and ONUJA (1984), GILL *et al.* (1985), HEIMSCH (1942), HUMMEL (1946), METCALFE and CHALK (1950). These authors reported the general comparative morphological variation in tracheary elements. The present paper analyses the nature of variation pattern along both the vertical and horizontal axes of the plant as well as the percentage composition of cellulose, lignin and hemicellulose along the vertical axis. The present report is a part of large project on the histochemistry of Nigeria timbers by the authors.

Material and Methods

Wood samples were obtained from a freshly felled tree at Sakpoba Forest Reserve (Lat. 06°04'N and Long. 05°52'E), Bendel State, Nigeria. Four transectional discs were cut at distances of 2 m intervals beginning from ground level (0, 2, 4 and 6 m level). Transradial longitudinal sections were cut out of each disc from pith at the various height levels for different analyses. One of such transradial longitudinal disc was used for histomorphological investigations. Thin slivers of wood were obtained at various linear distances of 2, 4 and 6 cm from the pith outwards in each of the four transradial longitudinal sections of the different height levels. Maceration of slivers of wood was carried out following GILL and ONUJA (1984). For each sample 100 measurements were made for each category of tracheary elements. A similar transradial longitudinal disc from each height level was reduced to saw-dust for chemical analysis. Saw-dust particle size was such that could pass a No. 60 sieve. The isolation of alpha-cellulose, holocellulose and lignin was carried out following the technique outlined by American Society for Testing and Materials ANSI/ASTM D1103-60, ANSI/ASTMD 1104-56 and ANSI/ASTMD 1104-56, respectively. Density of wood was determined at the various height levels following the gravimetric method of maximum moisture content as outlined by SMITH (1954). Wood compression strength at the various height levels was determined on "Cussions Compression/Tensile Strength Testing Machine" at the Department of Mechanical Engineering, University of Benin, Nigeria. To ensure a uniform distribution of the load over the wood sample, a uniform-sized oven-dried wood block (2 cm³) was cut out from each height level was used. Ultrasonic Pulse velocity was determined by using 'Pundit MKIII Test Machine' in the Structural Engineering Laboratory of the University of Benin, Benin City. Time taken for transmission of pulses through the test wood sample (2 cm³) at different height levels was read off from the Machine. Velocity was determined using the formula:

$$\text{Velocity} = \frac{\text{Distance}}{\text{Time}} \text{ m/s}$$

From Pulse velocity, 'Specific Acoustic Impedance' (SAI) was determined which is a product of pulse velocity and density.

Results and Discussion

Table 1 summarizes variations in Pulse Velocity, Specific Acoustic Impedance (SAI), Density and Compression Strength with plant height while Table 2 summarizes dimensions of fibre and vessel characteristics. Figures 1 and 2 are regression lines on the effect of plant height on density and wood chemical — components i.e. cellulose, lignin and hemicellulose.

The occurrence of small to medium-sized vessels (350-800 µm) have earlier been reported for this genus by GILL and ONUJA (1984), GILL *et al.* (1985), HEIMSCH (1942), HUMMEL (1946), KRIBS (1930), METCALFE and CHALK (1950). The present measurement of vessels (mean length and diameter of 417.40 µm and 109.92 µm), respectively, Table 2 differ from earlier reported dimensions (length and diameter of 545 µm and 290 µm) by GILL and ONUJA (1984). The present report of simple perforation on transverse endwalls and simple pits with no definite arrangement confirms the earlier report of GILL and ONUJA (1984).

Table 1

Relationship between pulse velocity, acoustic impedance, density and compression strength with plant height in *Khaya ivorensis* A. Chest.

Physical parameters	Plant height (m)			
	0	2	4	6
Pulse Velocity m/s	1.77	1.33	1.73	1.14
Specific Acoustic Impedance (SAI) kg/sq·m/s	1486.8	150.7	1453.7	946.2
Density g/cm ³	0.86	0.79	0.84	0.83
Compression Strength N	450	230	870	962

However, GILL *et al.* (1985) reported simple, round pits with reticulate arrangement for *K. grandifoliola*. The vessel length vary significantly along the vertical axis of the plant. Variation along the radial axis (pith outwards) was not significant for all the parameters tested. The report of medium-sized (900-1600 μm) nonseptate fibres confirms the earlier report from this taxon (GILL and ONUJA 1984). However, long-sized non-septate fibres have been reported for *K. grandifoliola* (GILL *et al.* 1985). Variations in fibre length and diameter along both the vertical and radial axes of the plant were not significant. F/V length ratio is 3.2 and did not vary significantly along both the vertical and radial axes. The presence of uniseriate and heterogenous rays 3-4 cm wide confirms the earlier report of PANSWIN (1933). However, GILL and ONUJA (1984), and GILL *et al.* (1985) have reported homogenous multiseriate rays for *K. ivorensis*, *K. senegalensis* and *K. grandifoliola*. Parenchyma in *K. ivorensis* is diffuse and paratracheal with an average length and diameter of 97.20 μm and 22.80 μm , respectively (Table 2). GILL and ONUJA (1984), reported parenchyma with an average length and diameter of 78.6 μm and 37.8 μm , respectively. The wood of *K. ivorensis* has an average density of 0.838/cm³ and is uniform along the bole length (Fig. 1). I has an average compression strength of 515.3N and is fairly hard and is comparable to *Azelia*, *Carya* spp. and *Peltogyne* spp. of commerce (DESCH and DINWOODIE 1981). Compression strength in *K. ivorensis* correlated positively with plant height Table 1. The chemical components of wood i.e. cellulose, lignin and hemicellulose vary along the vertical axis; but the variations were not significant at 5% level of probability. However cel-

Table 2

Fibre characteristics and vessel dimensions of *K. ivorensis* A. Chev.

Plant height (m)	Distance from pith (cm)	Mean fibre length (μ m)	Mean fibre diameter (μ m)	Mean lumen diameter (μ m)	Mean wall thickness (μ m)	Runkel ratio	Flexibility coefficient	Relative fibre length	Mean vessel length (μ m)	Mean vessel diameter (μ m)	Fibre/vessel length ratio
0	2	1120.6 +150.29	17.39+3.15	5.33+1.43	7.65+4.96	2.87	0.31	64.44	326.9 + 96.81	70.1 +27.39	3.43
	4	1412.70+197.65	19.44+4.89	9.75+5.44	4.95+0.61	1.01	0.50	72.67	381.10+128.80	51.83+39.14	3.70
	6	1425.52+152.51	23.29+3.86	11.14+2.78	6.07+1.34	1.08	0.48	61.21	377.74+131.28	116.77+51.88	3.77
	SUB \bar{X}	1319.60+168.45	20.04+3.96	8.33+2.71	6.33+2.30	1.65	0.43	66.0	311.91+118.80	79.56+39.49	3.63
2	2	1122.33+150.3	17.14+3.0	5.18+2.22	5.98+1.8	2.30	0.30	65.48	344.10+123.83	111.50+44.00	3.26
	4	1371.10+149.2	23.83+4.71	6.54+2.34	8.65+2.21	2.64	0.27	57.53	536.20+ 51.88	157.18+36.31	2.56
	6	1406.77+144.87	23.40+4.71	7.12+2.68	7.68+1.67	2.15	0.30	60.13	371.78+132.82	87.33+39.36	3.78
	SUB \bar{X}	1300.13+148.12	21.45+4.14	6.28+2.41	7.42+1.65	2.36	0.29	61.04	471.36+104.51	118.67+39.89	3.20
4	2	1335.2 +205.3	22.92+3.66	7.46+2.23	7.71+1.69	2.06	0.32	56.25	462.86+122.4	101.48+44.1	2.88
	4	1424.8 +198.3	21.28+5.37	7.36+3.61	7.07+2.08	0.92	0.34	66.95	553.11+126.16	117.0 +55.83	2.55
	6	1500.06+169.6	20.11+2.94	5.22+2.11	7.48+1.04	2.86	0.30	74.59	580.30+ 54.10	162.42+42.01	2.58
	SUB \bar{X}	1420.02+191.16	21.45+3.99	6.68+2.6	7.42+1.6	2.28	0.32	66.59	533.75+100.88	126.96+47.51	2.67
6	2	1229.09+174.3	21.43+4.29	8.03+2.82	7.65+4.60	1.90	0.37	57.35	328.3 +114.3	136.50+12.03	3.74
	4	1300.0 +140.82	23.66+2.28	11.44+1.34	11.26+2.28	1.96	0.48	54.94	341.71+ 72.65	128.14+11.69	3.80
	6	1193.0 +143.7	17.50+2.51	6.17+2.27	5.35+1.22	1.73	0.35	68.17	399.75+ 19.5	78.8 + 7.12	2.98
	SUB \bar{X}	1240.69+152.94	20.88+3.02	8.54+2.14	8.08+2.7	1.86	0.40	60.15	356.58+ 68.81	114.48+10.28	3.50
Tree	Mean	1320.11+165.20	20.95+3.78	7.25+2.60	7.29+2.06	2.05	0.06	63.48	417.40+ 98.29	109.92+34.23	3.26

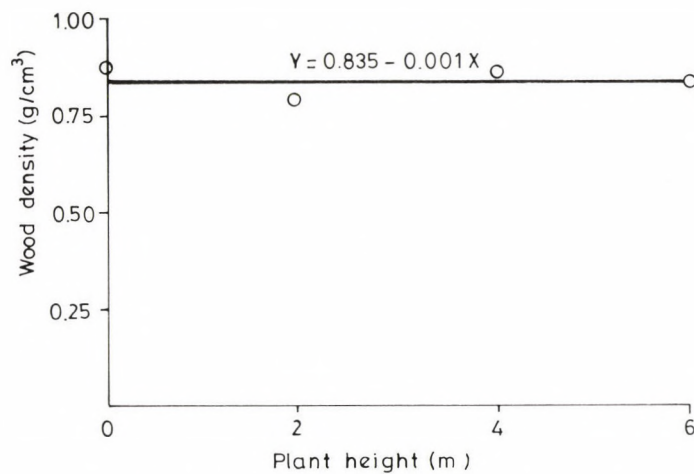


Fig. 1. Regression of wood density on plant height (*Khaya ivorensis* A. Chev.)

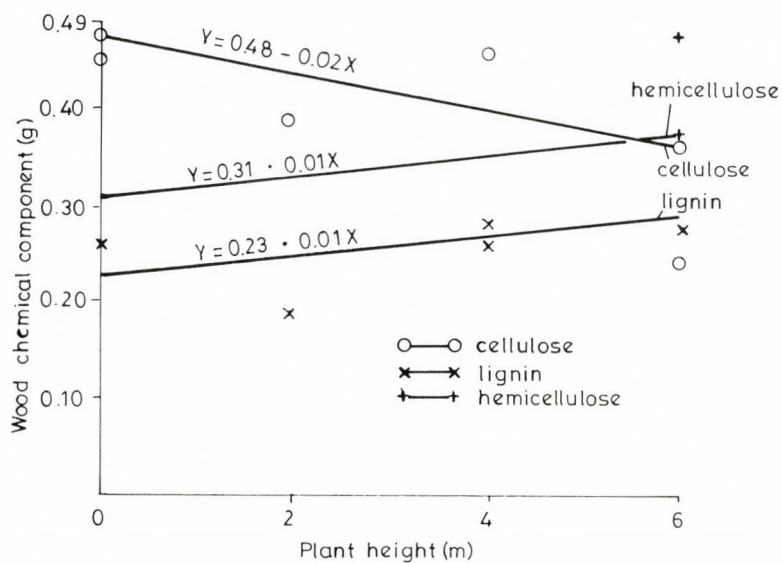


Fig. 2. Regression of wood chemical components on plant height (*Khaya ivorensis* A. Chev.)

lulose contents decrease as the plant height increases whereas both lignin and hemicellulose showed increase (Fig. 2). From Fig. 2 the mean percentage composition of cellulose, lignin and hemicellulose in *K. ivorensis* is within the standard range for these components as given by FENGEL and WEGENER (1984). Both pulse velocity and 'Specific Acoustic Impedance' showed negative correlation with plant height in *K. ivorensis*, but 'Specific Acoustic Impedance' showed positive correlation with compression strength (Table 1).

REFERENCES

- American Society for Testing and Materials (1979a): Standard test method for alpha-cellulose in wood. A.N.S.I./ASTM D1103-60, Philadelphia, 360-362.
- American Society for Testing and Materials (1979b): Standard test method for holocellulose in wood. A.N.S.I./ASTM D1106-56, Philadelphia, 364-367.
- American Society for Testing and Materials (1979c): Standard test method for lignin in wood. A.N.S.I./ASTM D1106-56, Philadelphia, 367-369.
- Desch, H. E., Dinwoodie, J. M. (1981): Timber — Its structure, properties and utilization. The Macmillan Press Ltd., London, 410 pp.
- Fengel, D., Wegener, G. (1984): Wood Chemistry Ultrastructure, Reactions. W. de Gruyter, Berlin, 613 pp.
- Gill, L. S., Onuja, J. E. (1984): Tracheary elements of some Nigerian woods (Meliaceae). J. Ind. Acad. Wood Sci. 15: 65-69.
- Gill, L. S., Lamina, B. L., Karatela, Y. Y. (1985): Histomorphological studies of the tracheary elements and the economic potentials of some tropical hardwoods. Sylvatrop. Philipp. For. Res. J. 10: 119-141.
- Heimsch, C. J. (1942): Comparative anatomy of the secondary xylem of the 'Criminales' and 'Terebenthales' of Wettesteia with reference to taxonomic groupings. Lilloa 8: 81-198.
- Hummel, F. C. (1946): The formation of growth rings in Entandophragma macrophyllum A. Chev. and Khaya grandifoliola C.DC. Emp. For. Rev. 25: 103-107.
- Keay, R. W. J., Onochie, C. F. A., Stanfield, D. P. (1964): Nigerian trees. I-II. Federal Dept. For. Res. Ibadan, 495 pp.
- Kribs, A. D. (1930): Comparative studies of the woods of the Meliaceae. Ann. Bot. 17: 724-738.
- Metcalfe, C. R., Chalk, L. (1950): Anatomy of Dicotyledons. I-II. Oxford Clarendon Press, London, 1500 pp.
- Panshin, A. J. (1933): Comparative anatomy of the woods of Meliaceae, subfamily Swietenioideae. Amer. J. Bot. 20: 639-668.
- Smith, D. N. (1954): Maximum moisture method for determining specific gravity of small wood samples. Rep. US For. Prod. Lab. No. 2014 Madison.
- Willis, J. C., Airy-Shaw, T. (1973): A dictionary of flowering plants and ferns. Cambridge Univ. Press, 1245 pp.

INTERNAL SEED STRUCTURE OF SELECTED VEGETABLE SPECIES

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In the years 1987-1989, a study on internal seed structure of selected vegetable species was conducted with the main goal to prepare some principal information on this topic, mainly for teaching purposes. This was achieved by preparing descriptions based on binocular microphotographs of blade hand-made sections of swollen seeds. The study included the following species: leek (Allium porrum L.), onion (Allium cepa L.), asparagus (Asparagus officinalis L.), pepper (Capsicum annuum L.), tomato (Lycopersicon esculentum Mill.), spinach (Spinacia oleracea L.), red beet (Beta vulgaris L.), head cabbage (Brassica oleracea L. var. capitata), radish (Raphanus sativus L. var. radicula), lettuce (Lactuca sativa L.), pumpkin (Cucurbita maxima Duch.), muskmelon (Cucumis melo L.), celery (Apium graveolens L.), carrot (Daucus carota L.), dill (Anethum graveolens L.), common bean (Phaseolus vulgaris L.), pea (Pisum sativum L.) and sweet corn (Zea mays L. var. saccharata). Seed of radish, head cabbage and lettuce, if destined for blade hand-made sections, should not imbibe longer than 2-3 hours at 20 °C; otherwise, they are very difficult to section due to the separation of their testas from the rest of the seed. Seeds of carrot, dill and celery were found to be difficult for hand sectioning because their embryos easily got separated from the endosperm. This occurs especially in small celery seeds. Staining the sections with 1% aqueous sour fuchsin was found to be effective, mostly because of its speed of reaction. The stain increased the contrast between an embryo and the rest of the seed and, therefore, could be highly recommended for seed sections staining for taking microphotographs, both colour transparencies and black and white pictures.

Keywords: seed structure, vegetable seeds, Compositae seeds, Chenopodiaceae seeds, Cruciferae seeds, Liliaceae seeds, Solanaceae seeds, Umbelliferae seeds, seed science teaching

Introduction

The seeds develop as a result of fertilization, which comes after successful pollination (GEORGE 1985). In the horticultural sense, a seed can be described as a dry dispersal unit, which develops from the ovule or ovule and associated tissues (ROBERTS 1972).

The angiosperm seed is usually comprised of the embryo, endosperm, perisperm and the testa or seed coat (BEWLEY and BLACK 1985).

Although mature seed embryos of monocotyledonous and dicotyledonous plants are significantly different: yet, their embryogeny is similar. They all come from proembryos (COPLAND and MCDONALD 1985). In most of dicotyledonous species the endosperm is formed, but is almost completely consumed during seed development. As a result of that, the mature seed is composed almost entirely of embryo. In the monocotyledonous species, however, the endosperm is not consumed but remains during seed development and comprises a major part of the mature seed (KOZŁOWSKI 1972).

Seed of vegetable species, in terms of their internal structure, could be divided into two main groups, i.e. the exalbuminous ones -- with little or no endosperm and the albuminous ones, which develop endosperm and perisperm (COPLAND and MCDONALD 1985).

During the courses of seed science and technology taught at Poznań Agricultural University, much attention has been paid to the internal structure of seeds of vegetable species. Although this topic, in its principles, has been a matter of intensive studies for over 60 years, detailed investigations of seed structure have been restricted to only a few agriculturally important seeds (DWARTE and ASHFORD 1982). Most of the works on this subject was done on a single species by plant anatomists or histologists (JONES 1927; GRUSHVICKII *et al.* 1963; JACOBSEN and PRESSMANN 1979). In the past, when research was done with the use of the binocular and light microscope, papers were accompanied with relatively few microphotographs. Nowadays, these facilities are used in seed research to a proportionally lesser extent. There is a trend in seed science towards increase use of scanning electron microscopy. This refers especially to works on seed coat patterns for taxonomic or identification purposes (SMITH 1982).

Vegetable seeds have always been considered as one of the more difficult objects for sectioning. Most of the seeds, when prepared for sectioning on microtomes, require complicated and time consuming processing (HOLUBOWICZ and GOFFINET 1988). These methods, although good for science and research, are of limited use for teaching purposes.

The present study was undertaken to prepare set of principal information on the internal seed structure of selected vegetable species based on microphotographs taken with the binocular microscope.

Material and Methods

Seeds materials. All seeds used for this study were bought from "CNOS" Poznań Horticultural Seed Company. Seeds of the following species were studied: leek, onion, asparagus, pepper, tomato, spinach, red beet, head cabbage, radish, lettuce, pumpkin, muskmelon, celery, carrot, dill, common bean, pea and sweet corn.

Sections and staining. All sections were done on freshly imbibed seeds. They were placed in water at 20 °C prior to sectioning. The length of soaking depended on the given species and varied from 2 to 24 hours. Excess water on the seed surface was removed with blotting paper. Sections were hand-made with a regular blade. They were first preliminary inspected with a magnification glass (5 x) and, then, the best sections were examined under the binocular (12.5-31 x). All the seed sections were left unstained and a few of them were also stained with 1% aqueous solutions of sour fuchsine for 5 minutes. After being stained, the objects were rinsed with water, dried out with a piece of blotting paper and reexamined under the binocular. For each species between 10 and 200 sections were completed.

Microphotographs. The sections, when accepted, were then examined by a photographer. Those with a best contrast amongst the principal seed parts, showing interesting details, were photographed. The pictures were taken with the use of a 1000 W halogen lamp and negative microfilm of high distribution and high contrast on using single, 6 cm x 9 cm, photographic membranes, 15 DIN or 20 DIN, respectively, produced in former East Germany. All pictured were taken with a special set for microphotographing designed for the "Practica" camera or for the single membranes. The films and the membranes were developed using the routine method. The pictures were printed on special photographic paper, produced in former East Germany.

Results

Leek, onion and asparagus (Plate I, Figs 1, 2, 3). Amongst these three studied species some similarities in their internal seed structure were found. The leek seed was more shrunken than that of the onion: its testa was thinner than the other two species. The embryo of leek was found to be less hooked and was thicker than the embryos of the other two species, and was similar to the onion seed. A relatively large volume of the leek seed was filled with endosperm (Plate I, Fig. 1). The onion seed had its testa shrunken to a smaller extent than the leek seed, its embryo was naturally hooked and a bigger part of the seed was filled with endosperm (Plate I, Fig. 2). The asparagus seed had rather a smooth, thick testa and thin, straight embryo. The asparagus embryo consisted of rather a short radicle and a long, single cotyledon (Plate I, Fig. 3).

Pepper and tomato (Plate I, Figs 4, 5). Both species had certain similarities in terms of their seed structure. The contrast between all the seed parts of both species was rather poor, so both their pictures were taken after staining the specimens. The best results were received when 1% aqueous sour fuchsine was used (Plate I, Figs 4, 5). In order to receive good sections of pepper and tomato, their seeds should not be soaked in water for longer than 24 hours.

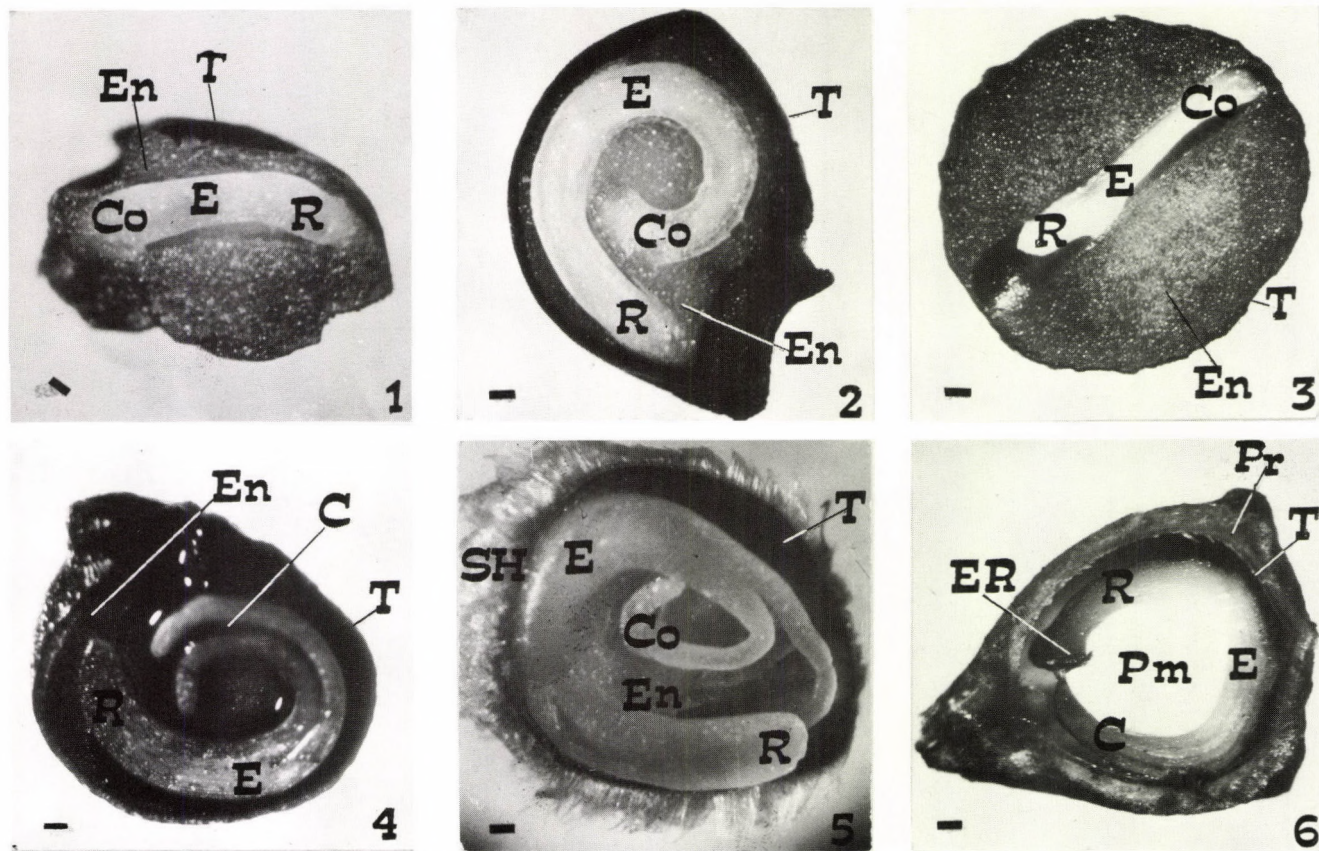


Plate I

Figs 1–6. Longitudinal sections of imbibed seeds of leek (1), onion (2), asparagus (3), pepper (4), tomato (5) and spinach (6).

Abbreviations: T – testa, E – embryo, En – endosperm, Co – a single cotyledon, C – cotyledons, R – radicle, SH – sericeous hair, Pm – perisperm, ER – endosperm remnants, Pl – preliminary leaves, LS – leaves, S – scutellum, Ce – coleoptile, Fl – first leaf, FN – first node, Cr – coleorhiza. Horizontal bars indicate the natural size of the seed

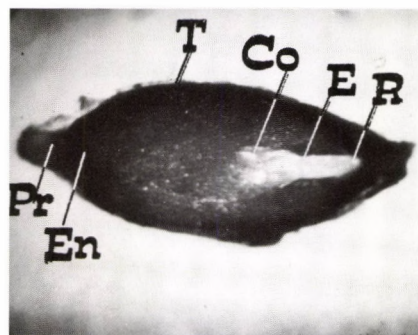
The pepper seed testa was thin and smooth (Plate I, Fig. 4), whereas the tomato testa was covered with sericeous hair (Plate I, Fig. 5).

The pepper embryo was rather thin, with the cotyledons adjoined to each other, located peripherally. The rest of the seed was filled with endosperm (Plate I, Fig. 4). The tomato embryo was found to be thicker than that of the pepper and had some variability in setting of an external cotyledon. In its classical position it was equal in size to the internal cotyledon; and located symmetrically. In two other settings it was either folded towards the testa or sticking closely to it; the cotyledon had its end under the radicle (Plate I, Fig. 5).

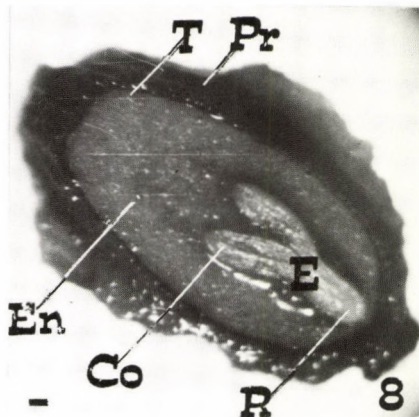
Spinach and red beet (Plate I, Fig. 6, Plate III, Fig. 13). Their internal structures are shown in figures 6 and 13, respectively. It was noticed that there a few similarities between them. The pericarps of spinach (Plate I, Fig. 6) and red beet (Plate III, Fig. 13) seeds were thick, and of a regular triangular or round shape for spinach and of an irregular shape for red beet. Their embryos were both well differentiated and their cotyledons and radicles could be easily distinguished. Between the embryo and the testa one could see the endosperm remnants. The center of the seed, for both species, was filled with snow-white perisperm (Plate I, Fig. 6, Plate III, Fig. 13). For good sectioning of these species a seed soaking time of up to 24 hours had been found to be preferable. Due to a high natural contrast among seed parts no additional staining was required.

Head cabbage and radish (Plate III, Figs 14, 16). The internal structures of seeds of both species were found to be very much alike. They had thin testas and embryos filled the reminder of the seeds, the embryo itself consisted of two large cotyledons and radicle (Plate III, Figs 14, 16). The shapes of the seeds, though, were slightly different. The head cabbage seeds were more spherical (Plate III, Fig. 14), whereas the radish ones were slightly flattened (Plate III, Fig. 16). When stained with 1% aqueous sour fuchisine, the seed parts of both species were found to have much better contrast.

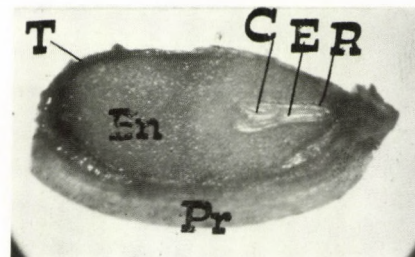
Lettuce (Plate III, Fig. 15). The seed was found to have a large embryo built of two big cotyledons and small radicle (Plate III, Fig. 15). At both ends of the seed small parts of the endosperm remnants were seen. It was found that for hand-made sectioning lettuce seeds could not be imbibe longer than 2-3 hours: otherwise, they were very difficult to section due to their testas separating from the rest of the seed. Additional staining



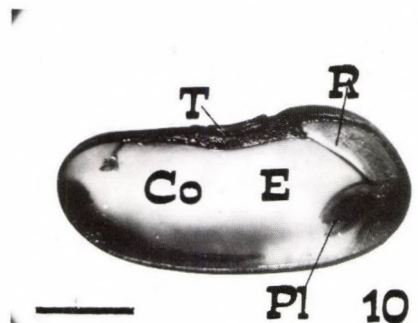
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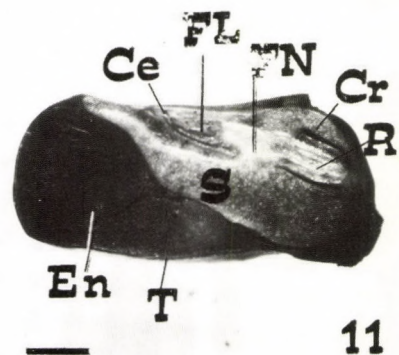
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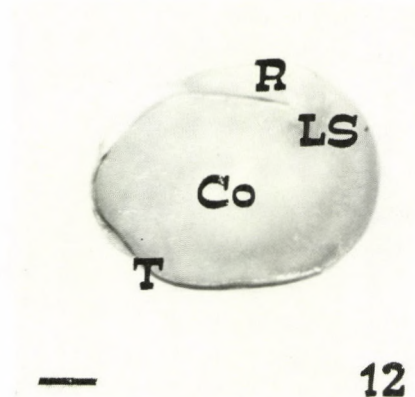
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10



11



12

Plate II

Figs 7–12. Longitudinal sections of imbibed seeds of celery (7), carrot (8), dill (9), common bean (10), sweet corn (11) and pea (12).

Abbreviations: see the explanation under Figs 1–6. Horizontal bars indicate the natural size of the seed

was also found to improve seed parts contrast, however, with so short inhibition the cotyledons were contiguous to each other (Plate III, Fig. 15).

Pumpkin and muskmelon (Plate III, Figs 17, 18). These two species were found to have many similarities. The seeds were composed of large embryos and testas (Plate III, Figs 17, 18). Two big cotyledons and a radicle were the most visible parts of their embryos. They were both located on the opposite sides of the seed. For obtaining good sections, no additional staining was necessary. The best sections were produced when seeds were soaked for 24 hours at 20 °C.

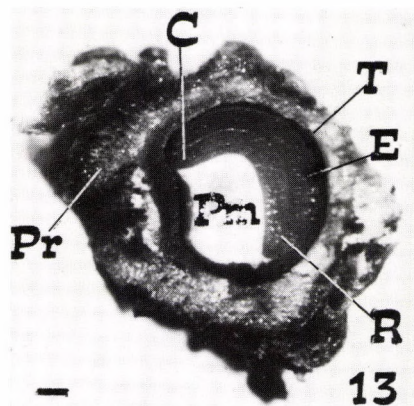
Celery, carrot and garden dill (Plate II, Figs 7, 8, 9). All the seeds of these three species were found to have poorly differentiated embryos, located towards one of the sharper ends of the fruit. Their embryos always comprised of two clearly seen parts, i.e. two symmetrical cotyledons and a single radicle located closer to the seed edge. The major part of the seed was filled with endosperm and surrounded by the testa grown together with the pericarp into one.

Amongst all the seeds studied, the celery seeds were found to be the most difficult for hand made sectioning mostly because of their small size (Plate III, Fig 7).

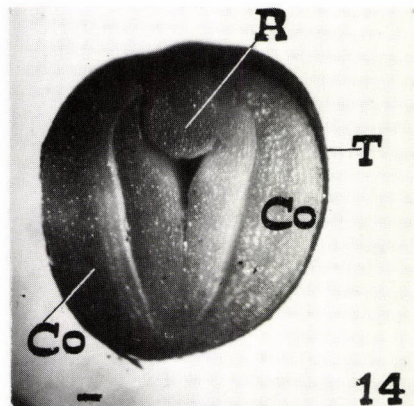
The embryos of carrot seeds were found to vary in length significantly. All sections of these three species had to be stained with 1% aqueous sour fuchsine due to their insufficient contrast for microphotographs.

Common bean and pea (Plate II, Figs 10, 12). The seeds of these two species, when swollen, were found to be the easiest for sectioning amongst all the vegetable species studied. Their seed had large cotyledons instead of the endosperm (Plate II, Figs 10, 12). By studying the common bean seed section microphotograph, it is possible to recognize a shoot axis with radicle and a pair of preliminary leaves. The common bean axis was located peripherally (Plate II, Fig. 10). When studying pea seed section microphotograph, a large cotyledon (the whole seed had two cotyledons) and a thin radicle could be seen (Plate II, Fig. 12). For pea and common bean microphotographing additional staining was necessary.

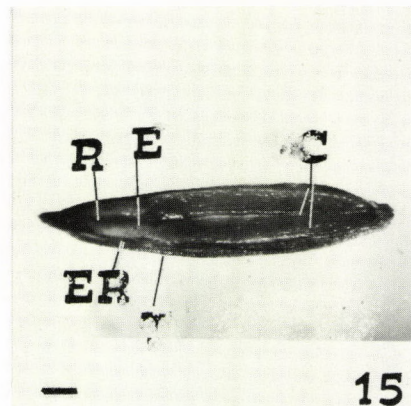
Sweet corn (Plate II, Fig. 11). The seed was found to consist of the strongly differentiated embryo, endosperm and testa (Plate II, Fig. 11). The embryo itself consisted of the following main parts: scutellum, first leaf, coleoptile, first node, radicle and coleorhiza. In order to improve the section's contrast for microphotographing staining the specimen with 1% aqueous sour fuchsine was found to be helpful.



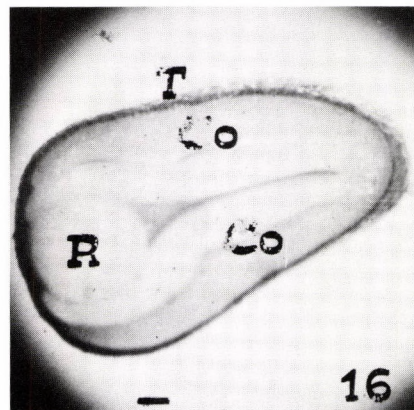
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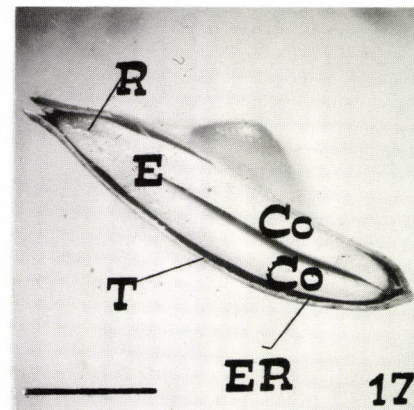
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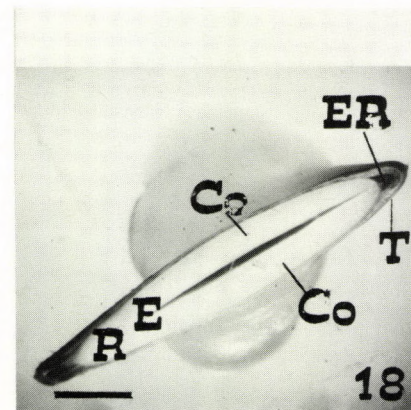
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16



17



18

Plate III

Figs 13—18. Longitudinal sections of imbibed seeds of red beet (13), head cabbage (14), lettuce (15), radish (16), pumpkin (17) and muskmelon (18).

Abbreviations: see the explanation under Figs 1—6. Horizontal bars indicate the natural size of the seed

Discussion and Conclusions

The results presented in this work are based on approximately 2000 individual seed sections.

It was found that the procedure tested by us for preparing seeds for classes was very effective and efficient. It was so, for both oral presentations and when a set of microphotographs was prepared. According to our present information, so far, no such information concerning vegetable seeds has been available. Most modern textbooks for seed biology are either based on detailed drawing or cite microphotographs from research journals. We suggest that the seed procedure, can be recommended because of its simplicity, speed in preparing, and low costs. It should therefore be used during seed science courses taught in both technical garden schools and agricultural universities. The method to prepare seeds for classes was tested during seed biology courses taught at our University in the years 1984—1991 and was quickly accepted by students.

The information received by us, when compared with the previous results of other authors, cited sporadically in various journals and textbooks, is in agreement with the data obtained by others (JONES 1927; TKACHENKO 1968; KRUG 1986).

The big similarities and slight differences between onion and leek seeds, e.g. the shrunken appearance of the seed coat, have already been reported by GRAY and WARD (1987).

Although the endosperm thin, double cell layer located in the lettuce seed could not be clearly seen on our microphotographs, it has already been known for over 60 years, as shown on the JONES' drawing (1927).

Seed structure of celery, carrot and garden dill has been a matter of rather vast and detailed study. Our pictures of all three species fit well with the information given by other authors (SZUJKÓ-LACZA 1978; TKACHENKO 1968; JACOBSEN and PRESSMANN 1979; DWARTE and ASHFORD 1982). The variability observed by us in the embryo length has been formerly reported by GRUSH-VICKII *et al.* (1963).

Seeds of radish, head cabbage and lettuce, if destined for blade hand made sections should not be imbibed for longer than 2-3 hours; otherwise their testas would become separated. Seeds of carrot, garden dill and celery were found to be difficult for hand sectioning for their embryos easily became separated from the rest of the seed. Staining the sections with 1% aqueous sour fuchsine was effective because of its speed of reaction. It

also increased the contrast between the embryo and the rest of the seed and could be, highly recommended, it is thought, for both colour transparencies and black and white pictures.

The procedure presented in this work to prepare seeds of selected vegetable species for classes of seed science or seed biology is simple, cheap and relatively easy to learn, even for somebody with little or no experience in seed biology. It can be, therefore, recommended mainly for students and teachers with limited numbers of hours for classes in this topic.

In conclusion it is necessary to emphasize the following:

1. Blade hand-made sections of swollen seeds of selected vegetable species showed well their internal structure and could be therefore recommended for teaching purposes.

2. When prepared for these purposes, seeds of radish, head cabbage and lettuce should not imbibe longer than 2-3 hours at 20 °C; seeds of carrot, dill and celery were difficult to use because their embryos easily got separated from the endosperm.

3. Staining the sections with 1% aqueous sour fuchsine increased the contrast between an embryo and the rest of the seed.

REFERENCES

- Bowley, J. D., Black, M. (1985): Seeds. Physiology on Development and Germination. Plenum Press, N. Y. and London.
- Copland, L. O. McDonald, M. B. (1985): Principles of Seed Science and Technology. Burgess Publ. Co. Minneapolis, Minnesota.
- Dwarte, D., Ashford, A. E. (1982): The chemistry and microstructure of protein bodies in celery endosperm. Bot. Gaz. 143: 164-175.
- George, R. A. T. (1985): Vegetable Seed Production. Longman, London and N. Y.
- Gray, D., Ward, J. A. (1987): A comparison of leek (Allium porrum) and onion (Allium cepa) seed development. Ann. Bot. 60: 181-187.
- Grushvickii, P. W., Agnayeve, J. J., Kuzina, J. F. (1963): On the varied quality of carrot seeds due to their embryo size. Bot. Zhur. 48: 1484-1489 (in Russian).
- Holubowicz, R., Goffinet, M. C. (1988): Polyethylene glycol embedment for histological studies of bean seed testa of low moisture content. Stain Techn. 63: 33-37.
- Jacobsen, J. V., Pressmann, E. (1979): A structural study of germination in celery (Apium graveolens) seed with emphasis on endosperm breakdown. Planta 144: 241-248.
- Jones, H. A. (1927: Pollination and life history studies of lettuce (Lactuca sativa). Hilgardia 2: 425-479.
- Kozlowski, T. T. (ed.) (1972): Seed Biology. Vol. I. Acad. Press, N. Y.

- Krug, H. (ed.) (1986): Gemüseproduction. Ein Lehr- und Nachschlagewerk für Studium und Praxis. Verl. P. Parey, Berlin und Hamburg, 211 pp.
- Roberts, E. E. (1972): Viability of Seeds. Syracuse Univ. Press.
- Smith, D. L. (1982): Seed development and structure. In: Thomson, J. R. (ed.): Advances in Research and Technology of Seeds. Part. 7. Centre for Agric. Publishing and Documentation. Wageningen, 62—86.
- Szujkó-Lacza, J. (1978): Hypostase, embryonic sac and endosperm in Anethum graveolens and in various families. Acta Biol. Acad. Sci. Hung. 29: 255—271.
- Tkachenko, N. M. (1968): Anatomical structure of seeds in vegetable crops. Trudy prikl. Bot. Genet. Selek. 40: 74—91 (in Russian).

PRELIMINARY STUDIES ON POSSIBLE Ni-HYPERACCUMULATOR PLANTS OF CUBA

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164 species of 66 selected genera belonging to 23 flowering plant families of the Cuban flora were tested for Ni-accumulation. All the tested plants — except one — are endemic to Cuba; two are limestone plants and 4 are facultative serpentinicolous species, the rest (158 species) are obligatory serpentine plants; 38 of them turned to be hyperaccumulators and further 19 species may be considered as moderate Ni-accumulators. Families of higher quantity of Ni-accumulators are: Asteraceae, Buxaceae, Euphorbiaceae and Rubiaceae.

Introduction

Chemical composition of serpentinicolous plants in Cuba was studied by ROSALINA BERAZAIN (1981) on 11 species living in the serpentine area of Loma Galindo in Matanzas province. Two of the studied species turned to be Ni-accumulator: Buxus flaviramea and Leucocroton havanensis (flavicans auct. cub. non Muell. Arg.). Loma Galindo belongs to the young serpentine areas of Cuba, its soils are rather poor in Ni, in comparison with the old serpentine areas, like Cajalbana in W-Cuba, and the serpentine mountains of East-Cuba (Nipe, Cristal, Moa and Baracoa), where important Ni-mines are found (BERAZAIN 1986; BORHIDI and MÚÑIZ 1986; BORHIDI 1973, 1974, 1975, 1988, 1991).

Our studies were concentrated to the serpentine endemics living on the mentioned old serpentine areas. 164 species were selected from the Herbarium of the Academy of Sciences of Cuba (HAC) and the Ni-content of the leaves were tested qualitatively with Waltham Ni-testing indicator paper. The first qualitative Ni-testing study was made by A. J. M. BAKER and R. REEVES on Cuban Phyllanthus-species collected by G. WEBSTER (Herb. of University of Davis) in 1991 and found several Ni-hyperaccumulators. In our present study the selected species belong to 66 genera of 23 flowering plant families representing a rather broad stock of the 920 serpentine endemics registered

in the flora of Cuba (BORHIDI 1988, 1991). Of the 164 species studied 2 are obligatory calcicolous (Leucocroton microphyllus, Pentacalia trineura), 4 species are facultative serpentine plants (Lyonia macrophylla, Callicarpa ferruginea, Rondeletia alaternoides ssp. alaternoides, Roigella correi-folia), the rest (158 species) are obligatory serpentinicolous plants.

In the evaluation of the test-reaction the following 5 degrees of Ni-indication were distinguished:

- = no colour reaction
- + = small pale crimson patches = slight accumulation
- ++ = continuous pale crimson colour = moderate accumulation
- +++ = intensive homogeneous crimson colour = hyperaccumulation
- ++++ = homogeneous intensive coloration with very intensive crimson points = strong hyperaccumulation

The results of the Ni-testing are found in the Table 1.

Of the 164 studied species 38 turned to be hyperaccumulators and further 19 species may be considered as moderate Ni-accumulators. Plants of "+++" and "++++" may be expected to contain more than 1000 ppm Ni, while plants with "+" and "++" indication may contain less.

Table 1

Qualitative analysis of possible Ni-hyperaccumulators of the flora of Cuba

1. Fam.: <u>Apocynaceae</u>		
Neobracea ekmanii Urb.	-	
Neobracea valenzuelana (A.Rich.) Urb.	-	
Rauvolfia salicifolia Griseb.	-	
2. Fam.: <u>Aquifoliaceae</u>		
Ilex shaferi Britt. & Wils.	-	
Ilex subavenia Alain	-	
3. Fam.: <u>Asteraceae</u>		
Feddea cubensis Urb.	-	
Pentacalia almironcillo (Maza) Proctor	+++	
Pentacalia cubensis (Greenm.)Borhidi	+++	
Pentacalia eriocarpha (Greenm.)Borhidi	++++	
Pentacalia leucolepis (Greenm.)Borhidi	+++	
Pentacalia moaensis (Alain)Borhidi	+++	
Pentacalia pachypoda (Greenm.)Borhidi	+	
Pentacalia polyphlebia (Griseb.)Borhidi	+++	
Pentacalia shaferi (Greenm.)Borhidi	+++	
Pentacalia trichotoma (Greenm.)Borhidi	+++	
Pentacalia trineura (Griseb.)Borhidi	-	
Senecio azulensis Alain	++++	
Senecio biseriatus Alain	+++	

Table 1 (contd.)

	<i>Senecio plumbeus</i> Griseb.	+++
	<i>Senecio rivalis</i> Greenm.	++
	<i>Senecio subsquarrosus</i> Greenm.	+++
	<i>Shafera platyphylla</i> Greenm.	-
4. Fam.:	<u>Bignoniaceae</u>	
	<i>Tabebuia pulverulenta</i> Urb.	-
5. Fam.:	<u>Boraginaceae</u>	
	<i>Bourreria pauciflora</i> O.E.Schulz	-
	<i>Cordia utemarkiana</i> Borhidi	-
6. Fam.:	<u>Buxaceae</u>	
	<i>Buxus crassifolia</i> (Britt.)Urb.	+++
	<i>Buxus flaviramea</i> (Britt.)Mathou	+++
	<i>Buxus gonoclada</i> Muell.Arg.	+++
	<i>Buxus leoni</i> Britt.	-
	<i>Buxus marginalis</i> (Britt.)Urb.	-
	<i>Buxus muelleriana</i> Urb.	-
	<i>Buxus olivacea</i> Urb.	-
	<i>Buxus pilosula</i> Urb.	+++
	<i>Buxus retusa</i> (Griseb.)Muell.Arg.	+++
	<i>Buxus rheedioides</i> Urb.	-
	<i>Buxus sclerophylla</i> Koehler	-
	<i>Buxus shaferi</i> (Britt.)Urb.	+
	<i>Buxus wrightii</i> Muell.Arg.	-
7. Fam.:	<u>Combretaceae</u>	
	<i>Bucida ophiticola</i> Bisse	-
	<i>Terminalia orientensis</i> Monach.	-
8. Fam.:	<u>Cyrillaceae</u>	
	<i>Purdiaea ekmanii</i> Urb.	-
9. Fam.:	<u>Dichapetalaceae</u>	
	<i>Tapura orbicularis</i> Ekm. ex Urb.	-
10. Fam.:	<u>Eriacaceae</u>	
	<i>Lyonia macrophylla</i> (Britt.)Ekm. ex Urb.	-
	<i>Vaccinium alainii</i> Acuña & Roig	-
11. Fam.:	<u>Erythroxylaceae</u>	
	<i>Erythroxylum coriaceum</i> Britt. & Wils.	-
12. Fam.:	<u>Euphorbiaceae</u>	
	<i>Bonania nipensis</i> Urb.	++
	<i>Chaetocarpus acuminatus</i> (Britt. & Wils.)Borhidi	-
	<i>Chaetocarpus oblongatus</i> (Alain)Borhidi	-
	<i>Croton borhidii</i> Muñiz	-
	<i>Croton miraflorensis</i> Borhidi & Muñiz	-
	<i>Euphorbia cubensis</i> Boiss.	+++

Table 1 (contd.)

<i>Euphorbia helenae</i> Urb.	+++
<i>Euphorbia munizii</i> Borhidi	+
<i>Euphorbia podocarpifolia</i> Urb.	+
<i>Gymnanthes recurva</i> Urb.	++
<i>Hyeronima nipensis</i> Urb.	-
<i>Leucocroton acunae</i> Borhidi	++
<i>Leucocroton cordifolius</i> (Britt. & Wils.)Alain	++
<i>Leucocroton cristalensis</i> Borhidi	++++
<i>Leucocroton dictyophyllus</i> Urb.	++
<i>Leucocroton ekmanii</i> Urb.	+++
<i>Leucocroton flavicans</i> Muell.Arg.	+++
<i>Leucocroton havanensis</i> Borhidi	+++
<i>Leucocroton linearifolius</i> Britt.	++
<i>Leucocroton microphyllus</i> (A.Rich.)Pax & Hoffm.	-
<i>Leucocroton moaensis</i> Borhidi et Muñiz	+++
<i>Leucocroton moncadae</i> Borhidi	++
<i>Leucocroton obovatus</i> Urb.	+++
<i>Leucocroton pachyphylloides</i> Borhidi	+++
<i>Leucocroton pachyphyllus</i> Urb.	+++
<i>Leucocroton saxicola</i> Britt.	+++
<i>Leucocroton stenophyllus</i> Urb.	+++
<i>Leucocroton subpeltatus</i> (Urb.)Alain	++
<i>Leucocroton virens</i> Griseb.	++
<i>Leucocroton wrightii</i> Griseb.	+++
<i>Moacroton cristalensis</i> (Urb.)Croiz.	-
<i>Moacroton leonis</i> Croiz.	-
<i>Moacroton lanceolatus</i> Alain	-
<i>Moacroton revolutus</i> Alain	-
<i>Moacroton trigonocarpus</i> (Griseb.)Croiz.	-
<i>Pera orientensis</i> Borhidi	+
<i>Phyllanthus cinctus</i> Urb.	-
<i>Phyllanthus comosus</i> Urb.	+++
<i>Phyllanthus chryseus</i> Howard	+++
<i>Phyllanthus discolor</i> Spreng.	++
<i>Phyllanthus excisus</i> Urb.	-
<i>Phyllanthus ekmanii</i> Webster	++
<i>Phyllanthus formosus</i> Urb.	++
<i>Phyllanthus grisebachianus</i> Muell.Arg.	-
<i>Phyllanthus incrustatus</i> Urb.	+
<i>Phyllanthus microdictus</i> Urb.	+++
<i>Phyllanthus orbicularis</i> HBK.	++++
<i>Phyllanthus phlebocarpus</i> Urb.	+++
<i>Phyllanthus pseudocicca</i> Griseb.	+++
<i>Platygyne obovata</i> Borhidi	-
<i>Platygyne triandra</i> Borhidi	-
13. Fam.: <u>Flacourtiaceae</u>	
<i>Xylosoma infestum</i> Griseb.	-
14. Fam.: <u>Melastomataceae</u>	
<i>Calycogonium moanum</i> Borhidi et Muñiz	-
<i>Miconia shaferi</i> Britt.	-
<i>Ossaea pauciflora</i> (Naud.)Urb.	-

Table 1 (contd.)

	<i>Ossaea rufescens</i> (Griseb.) Wr. in Sauv.	-
	<i>Pachyanthus neglectus</i> Borhidi	-
15. Fam.:	<u>Myrtaceae</u>	
	<i>Calycorectes moana</i> Borhidi	-
	<i>Calyptranthes punctata</i> Griseb.	-
16. Fam.:	<u>Polygonaceae</u>	
	<i>Coccoloba acunae</i> Howard	-
	<i>Coccoloba nipensis</i> Urb.	-
	<i>Coccoloba praestans</i> Borhidi	-
	<i>Coccoloba shaferi</i> Britt.	-
17. Fam.:	<u>Rubiaceae</u>	
	<i>Acrosynanthus latifolius</i> Standl.	-
	<i>Acrosynanthus minor</i> Urb.	-
	<i>Acunaeanthus tinifolius</i> (Griseb.) Borhidi	-
	<i>Antirhea shaferi</i> Urb.	-
	<i>Ariadne shaferi</i> (Standl.) Urb. ssp. <i>moaensis</i> Fernandez et Borhidi	+++
	<i>Casasia jacquinioides</i> (Griseb.) Standl.	-
	<i>Casasia nigrescens</i> (Griseb.) Wr. ex Urb.	-
	<i>Exostema myrtifolium</i> Griseb.	-
	<i>Exostema purpureum</i> Griseb.	-
	<i>Exostema revolutum</i> Borhidi et Fernandez	-
	<i>Exostema stenophyllum</i> Britt.	-
	<i>Guettarda ferruginea</i> Wr. ex Griseb.	-
	<i>Guettarda monocarpa</i> Urb.	-
	<i>Morinda moaensis</i> Alain	-
	<i>Neomazaea phialanthoides</i> (Griseb.) Kr. et Urb.	-
	<i>Phialanthus acunae</i> Borhidi	-
	<i>Phialanthus alainii</i> Borhidi	-
	<i>Phialanthus rigidus</i> Griseb.	-
	<i>Phyllomelia coronata</i> Griseb.	+++
	<i>Psychotria agustinae</i> Acuna	+
	<i>Psychotria cathetoneura</i> Urb.	++
	<i>Psychotria graminifolia</i> Urb.	+
	<i>Psychotria lopezii</i> Acuña et Roig	++
	<i>Psychotria moralesii</i> Acuña et Roig	+
	<i>Psychotria odorata</i> Wr. ex Griseb.	+
	<i>Psychotria pachythalla</i> Urb.	++
	<i>Psychotria rufovaginata</i> Griseb.	++
	<i>Psychotria shaferi</i> Urb.	++
	<i>Psychotria subulata</i> Wr. ex Griseb.	++
	<i>Psychotria thelophora</i> Urb.	+
	<i>Roigella correifolia</i> (Griseb.) Borhidi & Fernandez	-
	<i>Rondeletia alaternoides</i> Kr. et Urb.	-
	<i>Rondeletia lindeniana</i> A. Rich	-
	<i>Rondeletia miraflorensis</i> Fernandez & Borhidi	-
	<i>Rondeletia myrtacea</i> Standl. ssp. <i>brachyloba</i> Borhidi & Fernandez	-
	<i>Rondeletia odorata</i> Jacq. ssp. <i>bullata</i> Fernandez & Herrera	+

Table 1 (contd.)

	<i>Rondeletia pachyphylla</i> Kr. et Urb.	-
	<i>Rondeletia pycnophylla</i> Urb.	-
	<i>Schmidtottia elliptica</i> (Britt.)Urb.	-
	<i>Schmidtottia uliginosa</i> (Wernh.)Urb.	-
	<i>Scolosanthus lucidus</i> Britt.	-
	<i>Shaferocharis multiflora</i> Borhidi & Muñiz	-
	<i>Suberanthus stellatus</i> (Griseb.)Borhidi & Fernandez	-
	<i>Tocoyena cubensis</i> Britt. ex Standl.	-
18. Fam.:	<u>Rutaceae</u>	
	<i>Spathelia pinetorum</i> M.Vict.	-
19. Fam.:	<u>Sapotaceae</u>	
	<i>Manilkara mayarensis</i> (Ekm. ex Urb.)Cronq. ssp.	-
	<i>moaensis</i> (Gilly)Borhidi	-
20. Fam.:	<u>Theophrastaceae</u>	
	<i>Jacquinia moana</i> Borhidi	-
	<i>Jacquinia obovata</i> Urb.	-
21. Fam.:	<u>Thymelaeaceae</u>	
	<i>Daphnopsis oblongifolia</i> Britt. et Wils.	-
	<i>Lagetta pauciflora</i> Urb.	-
	<i>Linodendron aronifolium</i> Griseb.	-
22. Fam.:	<u>Turneraceae</u>	
	<i>Adenocubensis</i> (Britt. & Wils.)Arbo	+
23. Fam.:	<u>Verbenaceae</u>	
	<i>Callicarpa ferruginea</i> Sw.	-
	<i>Callicarpa lancifolia</i> Millsp.	-
	<i>Callicarpa oblanceolata</i> Urb.	-
	<i>Clerodendron nipense</i> Urb.	-

Discussion

The rather broad taxonomic and life-form variety of the studied material permits us to make some preliminary conclusions.

1) Ni-accumulation is an ability correlated mostly with highly specialized and/or evolved groups (Rubiaceae, Asteraceae, Euphorbiaceae, Buxaceae).

2) Ni-accumulation may be considered as an argument supporting the close taxonomic relation between Buxaceae and Euphorbiaceae.

3) Ni-accumulation is obviously not correlated with the accumulation of aromatic substances and volatile oils. Families like Lauraceae, Rutaceae,

Myrtaceae, Verbenaceae do not show Ni-accumulator plants, although these families developed a very high number of serpentine endemics. Even within one family are known closely related genera, one as positive Ni-accumulator without aromatic substances, the other as negative Ni-accumulator with aromatic substances. Such examples are: Leucocroton (Ni+), Moacroton and Croton (Ni-) in the Euphorbiaceae, Senecio and Pentacalia (Ni+), Eupatorium (Ni-) in the Asteraceae.

4) Ni-accumulation sometimes may be strongly correlated with taxonomic groups. E.g. the genus Leucocroton has 3 sections. Section Adeliocroton is completely Ni-, in the sect. Lasiocrotonopsis the species are moderate Ni-accumulators, while in the sect. Leucocroton almost all species are hyper-accumulators (BORHIDI 1992).

5) Ni-accumulation is obviously lacking in the families of high alkaloid content, like Apocynaceae, Erythroxylaceae, Solanaceae, although they develop important serpentine endemics.

6) Ni-accumulation is negatively correlated with families specialized to nutrient poor soils (like Ericaceae, Cyrillaceae, Melastomataceae), although many species of these families live on oligotrophic serpentine soils.

7) Lianes, vines are negative Ni-accumulators. Even in the families with many Ni-accumulators (Euphorbiaceae, Rubiaceae, Asteraceae) climber genera and species (Platygyne, Morinda moaensis, Chiococca cubensis, Lescaillea, Harnackia) do not accumulate Ni.

8) In the genus Buxus the Ni-accumulator species dry with yellow colour.

Closing remarks

The authors emphasize the preliminary character of this study and the very semiquantitative nature of the testing method. Although it is a quick method and useful for selecting the possible hyperaccumulators for a precise quantitative analysis from the great amount of the plant species adapted to serpentine habitats. The plants selected as hyperaccumulators will be analyzed by ALAN J. M. BAKER and ROGER REEVES in the Laboratory of the Massey University, New Zealand.

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REFERENCES

- Berazain, R. I. (1976): Estudio preliminar de la Flora serpentínicola de Cuba. Ciencias ser. 10. Bot. 12: 11-26.
- Berazain, R. I. (1981): Reporte preliminar de plantas serpentínicas acumuladoras e hiperacumuladoras de algunos elementos. Rev. Jard. Bot. Nac. Cuba 2: 48-59.
- Berazain, R. I. (1986): Algunos aspectos fitogeográficos de plantas serpentínicas cubanas. Feddes Repert. 97: 49-58.
- Borhidi, A. (1973): Kuba geobotanikájának alapjai (Fundamentals of the geobotany of Cuba). Diss. Dr. habil. Budapest, 292 pp.
- Borhidi, A. (1974): Kubában térképeztünk (Mapification in Cuba). In Hungarian. Gondolat Kiadó, Budapest, 245 pp. 116 tables.
- Borhidi, A. (1975): The effects of serpentine soils on tropical flora and vegetation in Cuba. XII. Intern. Bot. Congr. Leningrad, Abstracts I: 110.
- Borhidi, A. (1988): Efectos de la roca serpentina en la adaptación y evolución de la flora y vegetación tropical en Cuba. Acta Bot. Hung. 34: 123-174.
- Borhidi, A. (1991): Phytogeography and Vegetation Ecology of Cuba. Akadémiai Kiadó, Budapest, 858 pp.
- Borhidi, A., Muñiz, O. (1986): Phytogeographic survey of Cuba. II. Floristic relations and phytogeographic subdivision. Acta Bot. Hung. 32: 3-48.
- Brooks, R. R. (1987): Serpentine and its Vegetation. A multidisciplinary approach. Dioscorides Press, 454 pp.
- Brooks, R. R., Lee, J., Reeves, R. D., Jaffré, T. (1977): Determination of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J. Geochem. Explor. 7: 49-57.
- Reeves, R. D., McFarlane, R. M., Brooks, R. R. (1983): Accumulation of nickel and zinc by western North American genera containing serpentine tolerant species. Am. J. Bot. 70: 1297-1303.

SIMPLE IN VITRO PROPAGATION OF INSECTIVOROUS PLANTS

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A simple in vitro micropropagation method of 15 insectivorous plant species was described. The conclusion was that it is possible to reach optimal shoot and root formation on simple media without the addition of hormones, vitamins, and organic nitrogen source only by altering salt, agar, and sugar content and changing the pH value.

Comparisons were made for the speed of development, yield and period of time until blooming between insectivorous plants propagated in traditional glasshouse and the ones propagated in vitro.

Introduction

There are quite a few reasons of in vitro propagation of insectivorous plants:

1. Sustenance of rare and menaced species -- gene-bank function
2. Production of large number of plants in order to enable quick re-plantation when necessary
3. A couple of species are fairly decorative; can be grown as ornamental plants
4. Offers and easier way for hobby-gardeners and collectors to have these types of plants
5. Excellent demonstrative material for schools because they represent not only the morphology of insectivorous plants but give a general picture of aseptic in vitro cultures. It is possible to grow them without climate chamber or other equipments as the in vitro cultures of insectivorous plants are able to exist in good condition for 5-6 months without passing.

There were a couple of authors dealing with the in vitro propagation of insectivorous plants, e.g.: MOHAN RAM *et al.* (1972), DORE SWAMY and MOHAN RAM (1971) tested *Utricularia inflexa*, ADAMS *et al.* (1979) dealt with *Pinguicula moranensis* H.B.K., CARROL (1979) with *Pinguicula lutea*, WINTER

(1964), BEEBE (1980), PARLIMAN et al. (1982), HUTCHINSON (1984), MINOCHA (1985), with Dionea muscipula, WINTER (1964) tested Sarracenia species, CHANDLER and ANDERSON (1976), SIMOLA (1978) and KUKULCZANKA (1988) carried out experiments with Drosera. They all produced aseptic cultures of the above mentioned plants.

The majority of these authors obtained adventitious shoot and root formation by using different hormones, vitamins and organic nitrogen source.

We started our experiments in 1985 and carried out the simple micro-propagation of the following species: Drosera species and subspecies (sun-dews): D. capensis L., D. filiformis Raf., D. intermedia Hayne, D. dielsiana Exel., D. binata (Labill.) L., D. binata multifida ssp. dichotoma, D. binata multifida ssp. extrema, D. pygmaea DC, D. brevifolia Pursch, D. spathulata Labill., D. spathulata ssp. kansai, D. spathulata ssp. tanega, D. burkeana Planch., Dionea muscipula Ellis (Venus fly-trap), Pinguicula caudata Schlecht. (Synonym: P. moranensis H.B.K.) (butter-wort) Sarracenia flava L., S. purpurea L., S. alata L.

Material and Methods

The aseptic cultures of insectivorous plants can first of all be started from seed with the usual surface disinfecting methods.

Drosera and Pinguicula cultures can be started from mature seeds, Dionea were established with mature and green seeds, Sarracenia species from mature seeds or shoots.

Basal culture media contained MURASHIGE and SKOOG (1962) macro- and microelements' solution in original concentration (MS salts) and also its half or quarter strength variants. No vitamins was added. The optimal sucrose concentration was tested in 10, 20, 30 g/l variations. We attempted 2 g/l kasein-hydrolysate, 0.5 g/l peptone and 0.2 g/l L-glutamine as organic N supply. To adjust the optimal pH we tested media of the following pH-s: 5.0, 5.5, 5.7, 6.0, 6.5, 7.0. Factorial combinations of IAA (indole-3-acetic acid) at 0.25, 0.50, 1.00 mg/l and kinetin at 1.0, 5.0 and 10.0 mg/l were examined. In order to find the optimal agar (Oxoid) concentration we studied the following amounts: 5.5 g/l, 6.0 g/l, 6.5 g/l, 7.0 g/l, 7.5 g/l, 8.0 g/l.

Treatments were replicated minimum 10 times.

All cultures were incubated at $23 \pm 0^\circ\text{C}$ under warmwhite fluorescent light (6000 lux) on a 16+8 day-night cycle.

The plants were passed each 6-8 week but they could be kept in the same medium even 5-6 months.

The suitable-size in vitro plants were planted in peat (Novobalt)-powdered charcoal mixture (9:1) pH = 4.5-5.5. The peat needs to be completed with 30% garden-soil and Ca-substitute (Futor) in case of Pinguicula. In order to maintain the acclimatisation the plants were kept in closed plastic boxes which ensured the vapour content for two weeks, then were out to climatized glasshouses, freely.

Results and Discussion

At the very beginning of the test series we found that the different insectivorous species and subspecies behave very similarly to each other and can effectively be micro-propagated using simple medium.

As we have mentioned in the introduction, the majority of the experts dealing with micro-propagation of insectivorous plants obtained their results using hormones, organic nitrogen and other supplementary materials. We reached the required shoot and root formation with the alteration of the salt, sucrose and agar content of the medium and with changing the pH value.

The optimal shoot formation and their quality was especially influenced by the hardness of the medium (agar content). In case of all species tested by us the combination of kinetin and IAA had a couple of negative influences like excess shoot formations, distortion and vitrification. With the exception of one species it is either unnecessary or even harmful to use organic N supplies that were tested by us. It is also needless to add vitamins. In all species the half strength MS salts and 20 g/l sucrose in the basic medium ensure the optimal growth of plants.

Further results will be given according to species: Dionea muscipula: half strength MS inorganic salts, 20 g/l sucrose, 8.0 g/l agar, pH 5.5 without hormones or other additives. To start the culture the best we found was to use sterile seed from sterilized closed seed capsule.

Germination is rapid, the 2 cm plants already have shoots even in hormone-free medium. If the medium contains cytokinin, the number of shoots is greater but there are malformations and vitrifications. Therefore it is more difficult to plant out these plants.

It is very important to care about the hardness of the medium in case of Dionea -- the vapour-content of the nursing-pot should be reduced. Quality can be improved this way, too.

It is advisable to pass them every 6 weeks -- then plants can be cut into 9-10. Thus it is possible to have 4-5000 plants/year of a single stem (Fig. 1).

With the traditional propagation we could only have maximum 100 seeds from the one inflorescence having 15 flowers. Under normal conditions we can observe adventitious shoots only in mature plants, therefore it is not possible to increase the number of plants significantly though they are grown until their blooming through a 3-year period. In vitro Dionea plants bloom in the second year.

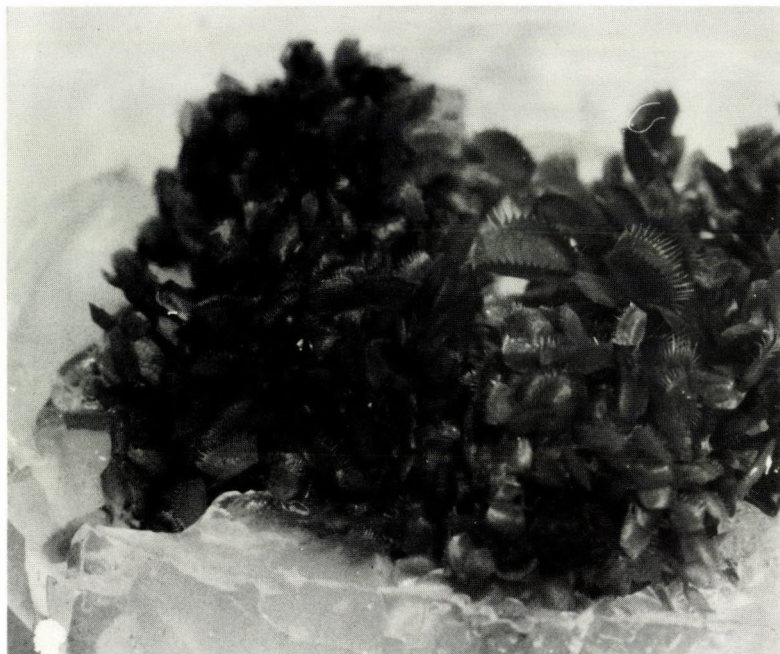


Fig. 1. Dionea muscipula in vitro plants

In Drosera species the following medium was optimal: 1/2 strength MS inorganic salts, 20 g/l sucrose, 6.0 g/l agar, pH = 5.5 without hormones or additives. The only exception is Drosera spathulata which would need 2 g/l kaseinhydrolysate to get a forceful growth.

All Drosera species should be started from mature seed; to start from rosetta due to lack of sterilisation. Seeds will germinate on light and in sterile medium after two weeks. Shooting is uniform. Growth gets quicker and quicker and in the majority of species rosetta is formed in 2 months time. The stalked glands are well developed, but the excretion of the adhesives is low.

It is possible to get normal size plants in vitro in case of a part of Drosera species; 5-7 months after sowing, 3 months after passing (D. pygmea, D. spathulata, D. burkeana, D. intermedia, D. brevifolia). There are quite a couple of shoots forming even without cytokinine addition. When passing in every 2-3 months, it is possible to have 7-10 shoots with homogeneous size-distribution. These plants usually have their own roots thus it is possible to plant out them.



Fig. 2. More than 100 Drosera binata plantlets in one flask — without hormones

In the other groups of Drosera species the in vitro plant will not reach the normal plant size (D. binata, D. capensis). The in vitro shoot forming is even more intensive in this case (Fig. 2). It is possible to get 20 homogeneous shoots each 2-3 months. In Drosera species it is advisable to separate the shoots without cutting, by the help of two dental forceps. The in vitro propagated Drosera plants can be planted to glasshouses or passed any time of the year, but it is necessary to ensure additional light in winter time. It needs to be emphasized that practically we can get as many plants as we wish, fairly cheap and with little effort. It is much quicker to produce this way Drosera species that are able to bloom than in glasshouse circumstances.

Plants that bloom after 1-1.5 years in glasshouse, will bloom after 6-8 months and the ones that bloom after 2-3 years, will bloom after 8-12 months after planting out. In the majority of species we can observe in vitro bloom-formation after 6-8 months.

The optimal composition of the medium for Pinguicula caudata is: 1/2 strength MS inorganic salts, 20 g/l sucrose, 6.0 g/l agar, pH = 6.5, without



Fig. 3. *Pinguicula caudata* in vitro plants

hormones and other additives. To start the culture the best we found was sterile seed from sterilized closed seed case. Growth after gathering is fairly slow.

If we pass the 5-7 cm plants to new medium, the growth becomes protracted. If we cut them to 2-4 pieces, there will be intensive shoot-formation together with plant regeneration without any hormones (Fig. 3). After 2-3 months it is possible to separate 5-8 shoots from the original plant. We can get similar results when putting the well-grown lower leaves to medium, but in this case the process is slower.

As a comparison, we carried out the evaluation of traditional horticultural propagation. In the latter case it was possible to grow 150 plants from the seeds gained from the case within the 3 years-period until blooming -- including the stem-division and propagation. With the in vitro propagation carried by us we were able to produce 1000 plants with continuous planting. For planting the 2-3 cm plants are most suitable especially the ones with good condition roots. It is very important to ensure the required Ca supply in the planting medium, and also to set the appropriate pH (6.5-7.0).

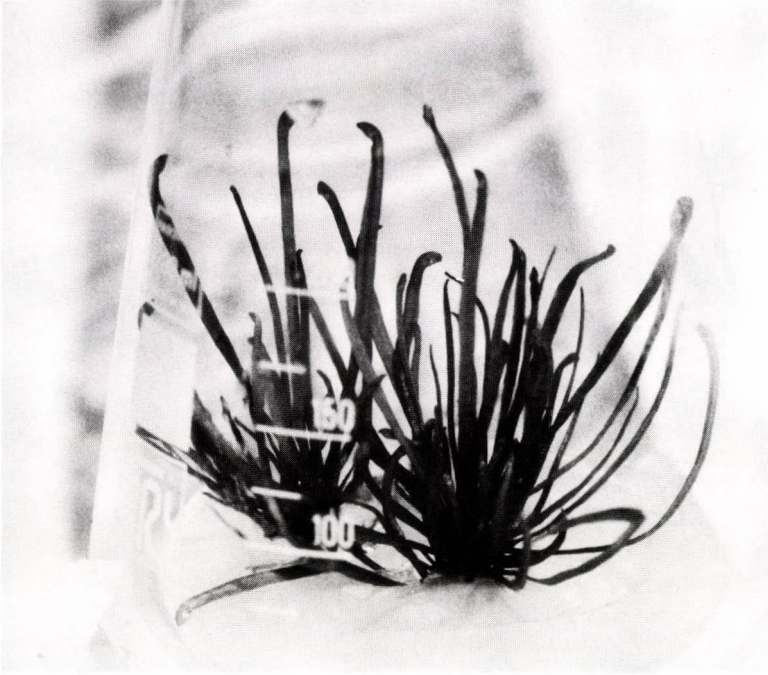


Fig. 4. Sarracenia flava in vitro plants

For Sarracenia flava, S. purpurea, S. alata the following medium was optimal: 1/2 strength MS inorganic salts, but original quantity of (25 mg/l) NaFe — EDTA, 20 g/l sucrose, 6.0 g/l agar, pH = 5.7 without hormones or additives. To start the culture mature and fallen seed is most suitable, but it is possible to start from shoots. Germination of the seeds is rather slow even in vitro. The germinated plants will reach a 7 cm state in 3-4 months. There are 1-2 shoots already present at this stage and in the further passing there are 6-8 shoots that can be regularly separated (Fig. 4).

Thus it is possible to have 3000 plants/year from one original plant. Traditional horticultural propagation is rather difficult. In our experience gathering starts 6 weeks after sowing. Only 0.2% of the seeds will germinate. Gathering will continue for 1.5-2 years. The percentage of germination will reach 70% by then. Natural shoot-formation starts only in the second year, with 2-3 shoots annually. The advantages of in vitro propagation can clearly be seen in case of Sarracenia and contribute to their popularity as ornamental plant to a large extent.

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REFERENCES

- Adams, R. M., Langhans, H. R. W., Koenigsberg, S. S. (1979): Carnivorous plants underexploited for indoor culture. Hortsci. 14: 678.
- Adams, R. M., Koenigsberg, S. S., Langhans, R. W. (1979): In vitro propagation of the Butterwort Pinguicula moranensis H.B.K. Hortsci. 14: 701–702.
- Beebe, J. D. (1980): Morphogenic responses of seedlings and adventitious buds of the carnivorous plant Dionea muscipula in aseptic culture. Bot. Gaz. 141: 396–400.
- Carrol, B. (1979): Propagating Pinguicula lutea. Carniv. Plants Newsletter 8: 58.
- Chandler, G. E., Anderson, J. W. (1976): Studies on the nutrition and growth of Drosera species with reference to the carnivorous habit. New Phytol. 76: 129–141.
- Dore Swamy, R., Mohan Ram, H. Y. (1971): Studies on growth and flowering in axenic cultures of insectivorous plants. Induction of flowering and development of flower in Utricularia inflexa. Z. Pflanzenphysiol. 65: 315–325.
- Hutchinson, J. F. (1984): In vitro propagation of Dionea muscipula ELLIS (Venus fly trap). Sci. Horticult. 22: 189–194.
- Kukulczanka, K. (1988): Anwendung von in vitro Kulturen bei aktivem Schutz von Pflanzenarten. Wiss. Zschr. Friedrich Schiller Univ. Jena, Beitr. z. Phytotax. 37: 82–85.
- Minocha, S. C. (1985): In vitro propagation of Dionea muscipula. Hortsci. 20: 216–217.
- Mohan Ram, H. Y., Harada, H., Nitsch, J. P. (1972): Studies on growth and flowering in axenic cultures of insectivorous plants. Effects of photoperiod, Ethrel, morphactin and a few other growth substances and metabolic inhibitors on Utricularia inflexa. Z. Pflanzenphysiol. 68: 235–253.
- Murashige, T., Skoog, F. S. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum 15: 473–497.
- Parlman, B. J., Evans, Ph. T., Rupert, E. A. (1982): Tissue culture of single rhizome explants of Dionea muscipula ELLIS ex L. the Venus fly trap, for rapid asexual propagation. J. Amer. Soc. Hort. Sci. 107: 305–310.
- Simola, L. K. (1978): Dipeptides as nitrogen sources for Drosera rotundifolia in aseptic culture. Physiol. Plant. 44: 315–318.
- Winter, C. L. (1964): Notes on cultivation of insectivorous plants in vitro. Bull. Torrey Bot. Club 91: 412–413.

EFFECTS OF CARBON SOURCES ON THE MORPHOLOGY AND STRUCTURE OF SCENEDESMUS ACUTUS MEYEN

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Die durch verschiedene Kohlenstoff-Quellen (CO_2 , Äthanol, Glucose, Melasse) induzierte morphologische und strukturelle Variation eines neuen Stammes von Scenedesmus acutus Meyen ("Mono") wurde in statischen und intensiven Kulturen untersucht.

Das Verhältnis zwischen tetra-, di- und monodesmoiden Coenobien variiert in Abhängigkeit von den Kulturbedingungen: in statischen Kulturen herrschen die tetradesmoiden, in intensiven Kulturen die monodesmoiden Coenobien vor. Oxygen-Mangel bedingt eine Zunahme der tetradesmoiden Coenobien, während die Gegenwart von Melasse und Glukose ihre Häufigkeit herabsetzt. Organische Kohlenstoff-Quellen vergrößern die Zelldimensionen.

Die verschiedenen Kohlenstoff-Quellen führen zu tiefgreifenden Veränderungen der Zellstruktur. So verursacht Glukose eine betonte Verdickung der Zellwände, Melasse eine Denaturierung der Chloroplastenstruktur durch starke Einlagerungen von Stärke, während Äthanol eine Schwellung und Isolierung der Thylakoide, sowie die Bildung von Vesikeln anstelle von Cristae in den Mitochondrien hervorruft.

Introduction

The better understanding of the role of carbon sources in the life of algae has been a thrilling task for almost a century (BEIJERINCK 1890; ARTAR 1899; SENN 1899; GROSSMANN 1921; HELLEBUST, YU-HUNG LIN 1978; VENKATARAMAN, BECKER 1985). The interest was uninterrupted, perhaps, because carbon as a nutrient element is generally available in limiting amount for the autotrophic plants (GOLDMAN et al. 1972; BECKER 1982; VENKATARAMAN, BECKER 1985) and due to the strive to perfectionate algal culture. Indeed, to ascertain the trophicity, the nutritional requirements of a new, not yet known algal species (strain), its morphological and structural characteristics brought about by different environmental factors was not a worthless experiment, and it is still interesting nowadays (WEIER et al. 1970; NAGY-TÓTH 1987).

Material and Methods

The alga herein dealt with was isolated (May 15th, 1972) from a yellowish-green mucilaginous mass adhering to the inner side of the concrete wall, at the level of the water surface of the spring called "Büdöskút" (pH 6.5-7.0; temperature 14 °C) surrounded by arable lands belonging to the Mono village (Sălaj District). It was identified according to GRINTZESCO (1902), HORTOBÁGYI (1941, 1957, 1978) and UERKOVICH (1966) as *Scenedesmus acutus* Meyen very close to f. *alternans* Hortob.

The unialgal stock (static) cultures were successively maintained and/or in different solid (agarized) or liquid media (usually KNOP-PRINGSHEIM, Benecke-K₂HPO₄, WITSCH and KUHLL-LORENZEN), in 100 ml Erlenmeyer flasks (containing nearly 30 ml of nutrient media). The experimental batch cultures were performed in Vladimirova-Semenenko's vessels provided with frit-ended bubbling tubes made of "Rasotherm" glass (NAGY-TÓTH 1972). Their total volume was nearly 275 ml and contained about 225 ml of nutrient solution. In the first experimental series the Kuhl-Lorenzen's solution was used (prepared from stock solutions). Its pH was adjusted (with a mixture of KOH + NaOH 20% in the proportion of 3.1 + 1) to the value of 6.7-7.0 before autoclaving (at 110 °C, 1 h). The organic carbon sources were added to this basal medium after autoclaving. The following variants were set up:

- (a) air + 1.5-3% CO₂ (control),
- (b) air - O₂ (retained from the bubbling air),
- (c) air - O₂ + 1.5-3% CO₂,
- (d) air + molasses,
- (e) air + molasses + 1.5-3% CO₂,
- (f) air + glucose + 1.5-3% CO₂,
- (g) air + ethanol,
- (h) air + ethanol + 1.5-3% CO₂.

Another series of experiments was performed in Kuhl-Lorenzen's, Knop-Pringsheim-Feldy's and Tamiya-urea EH's complete solutions. The bubbling air was sterilized by passing through a 5% KMnO₄ solution, distilled water and cotton wool filter. The C₂ from the sterilized air was retained (for variants (b) and (c)) by passing through a 10% pyrogallol solution (prepared according to BLAZHENOVA *et al.* 1957) and followed by 2 N H₂SO₄; or instead air pure nitrogen was used.

All the variants were inoculated with 25 ml of algal suspension made of a subculture grown in KUHLL-LORENZEN's solution. The initial cell density was 40 cells/μl, which corresponded to an optical extinction of 0.005 (measured in cuvette of 10 mm, but related to that of 1 mm, at 597 ± 10 nm by means of a FEK-56M photocolormeter). These suspensions were poured into Vladimirova-Semenenko's vessels exposed to 10000 + 10000 lux (illuminated bilaterally by fluorescent tubes of 40 W "Daylight") for 13 hrs per day. Cultivation lasted 7 and 12 days, respectively, concluding with the end of the log phase in control (grown in KUHLL-LORENZEN's solution).

Morphological variations (morphoses) were investigated in light microscope by drawing 80 coenobia or cells taken randomly with a camera lucida (Pls I-III).

The electron microscopical observations were carried out by employing the standard technic used for green algae in the Electron Microscopy Laboratory of the Cluj University (PÉTERFI *et al.* 1979, 1988). The algal material was sampled from the same experimental variants (a-h) recorded before, after a growing time of 7 days.

Results and Discussions

Morphological variations of coenobia and cells (pleomorphism)

The morphological changes occurring in Scenedesmus species during their developmental stages, or arising under various natural and experimental conditions as well (pleomorphism), have been thoroughly examined. Beyond the taxonomical significance of this phenomenon, the overwhelming proportion of one or another coenobial stage within the same species can provide information on the preponderance or limitation of an environmental factor. During the last decades the adequate changes of coenobial stages ("Coenobiumformwechsel") were actually used to test different substances or analyse waters and soils. Thus, it was evidenced that the coenobial structures ("Coenobiagram") of different strains (276-4e, Müggelsee, Tjukemeer) of Scenedesmus quadricauda (Turp.) Bréb. varied sensitively according to nutrient solution concentrations and phosphorus content (OVERBECK, STANGEBURSCHE 1966; STEENBERGEN 1978), to nitrate availability (GAVIS *et al.* 1979). Moreover, it was found that in S. quadrispina Chod. the proportions among the one-, two-, four- and eight-celled coenobia have been significantly changed by auxin and various wheat extracts (NAGY-TÓTH 1964); in Scenedesmus strain IUCC 1591 not only the number of cells in coenobia varied, but even their spininess depending on NH_4^+ concentration along a river flow (TRAINOR *et al.* 1976).

The overall habitus of the populations (facies culturalis) of Scenedesmus acutus Meyen (strain Mono) developed in these cultures reflects the effects of the composition and ratios in nutrient elements (e.g. C, N, P) of the media used, the carbon sources added and, of course, it depends on their age and growth stage.

In static cultures grown in KÜHL-LORENZEN's solution (subcultures), four-, two- and one-celled (scenedesmoidic, didesomoidic, monodesmoidic) coenobia were rather frequent, their characteristically alternating adjoined cells corresponding to the diacritic attribute of S. acutus Meyen f. alternans Hortob. (HORTOBÁGYI 1941) (Pl. I, Figs 1--4). Nevertheless, besides one-row coenobia, as well as palmelloid thalli were also present (Pl. I, Fig. 1). The cells looked broadly-fusiform (marginal cells), or fusiform (inner cells), sometimes cylindrical, slightly asymmetrical (lagenaria-like, Pl. I, Figs 2, 3), with gradually narrowed or bluntly rounded poles (Pl. I, Fig. 1) with short papilla on the apexes, which may be absent. The ratio between the length and breadth of the cells was 2.67:1, while in natural

populations it amounted to 3.21:1 (UHERKOVICH 1966). The chloroplasts were extended over $3/4$ of the inner cell wall, having lobulated margins and bearing always well visible pyrenoids of variable size.

In batch cultures similarly grown in KÜHL—LORENZEN's solution, the one-celled forms were overwhelming, but four-celled coenobia of alternating cells were not infrequent. The cells were broadly-fusiform or even ellipsoidal having short poles and tiny papillated apices. Aberrant forms appeared rarely (Pl. I, Figs 5--15). Chloroplasts trough-shaped, moderately developed, with sinuous margins and variable pyrenoids. In KNOP—PRINGSHEIM—FELFÖLDY's solution, the coenobia were weakly organized; the population was almost monodesmoidic, displaying, here and there, very loosely adjoined didesmoidic coenobia, too (Pl. I, Figs 16--24). The shape of cells was more variable than those in KÜHL—LORENZEN's solution, mainly asymmetric with irregularly rounded poles and very short papillae or lacking any emergences. The size of cells was, generally, larger than in KÜHL—LORENZEN's solution, which could be due to the soil extract (as organic ingredient) present in KNOP—PRINGSHEIM—FELFÖLDY's solution. The chloroplasts looked thin, granulated and perforated covering almost entirely the inside of cells. Pyrenoids were small. In TAMIYA's solution singular cells were preponderant, four-celled coenobia were scarce, but the two-celled ones were slightly more numerous than in the previous culture. The cells in coenobia were weakly attached; no typical scenedesmoids could be seen. The shape of cells also varied, but fusiforms predominated, and transient forms could be seen (Pl. I, Figs 25--31). Their ends narrowed gradually in a small papilla. Chloroplasts were well evolved. Pyrenoids were characteristically small. Noteworthy was the presence of the mother cell wall remnants in these cultures (Pl. I, Figs 29--31). The average cell breadth was lower than in the other two variants (Table 1).

The aspect of the populations grown in O_2 -less cultures, either with or without CO_2 supply (variants (b) and (c)), generally, resembled that which shared O_2 -containing air and excess CO_2 (control). Even the dimensions were close to that. Nevertheless, the mature coenobia as well as cells were somehow longer, the structure of coenobia more lax (Pl. II, Figs 32--37) and, consequently, the poles remained more free, the apices looking elongated enough and displaying well visible papillae. This aspect differs from those described by earlier authors, according to whom O_2 favors one-celled, as well as dactylococcoid stages in some coenobial Chlorococcacean algae (SENN 1899; GRINTZESCU 1902; CHAMBERS 1912). The average cell sizes

Table 1

Cell sizes of *Scenedesmus acutus* Meyen, strain "Mono" grown in static and batch cultures in different nutrient solutions (in μm)

Nutrient solutions \ Cell sizes	Limits	Averages
Static cultures (inoculum) in Kuhl-Lorenzen's solution	3.50— 6.50 x 6.50—10.80	4.39 x 8.31
Batch cultures in Kuhl-Lorenzen's solution	2.50— 6.50 x 5.00—11.50	4.40 x 8.19
Knop-Pringsheim-Felföldy's solution	4.20— 7.00 x 8.30—11.00	5.20 x 9.31
Tamiya urea EH solution	2.80— 6.00 x 7.50—12.50	4.21 x 9.31
Air + CO ₂ *	3.00— 5.25 x 9.00—12.50	4.04 x 10.81
Air - O ₂	2.75— 5.25 x 7.00—13.50	3.95 x 10.57
Air - O ₂ + CO ₂	2.75— 6.00 x 9.50—16.25	4.16 x 13.14
Air + molasses	5.00— 7.75 x 8.00—17.50	7.28 x 11.14
Air + molasses + CO ₂	3.75— 7.75 x 10.00—18.00	5.73 x 13.48
Air + glucose + CO ₂	3.75—13.25 x 8.75—12.75	7.53 x 11.25
Air + ethanol	3.25— 6.00 x 8.00—13.50	4.27 x 10.87
Air + ethanol + CO ₂	4.00— 8.50 x 8.25—12.50	5.32 x 10.06

*For all variants Kuhl-Lorenzen's solution was used.

in the cultures supplied with CO₂ were larger than in the O₂-less ones. It seems that the excess of CO₂ stimulated the increase of chloroplasts (Pl. II, Figs 38—42), which incidentally have perforations (Pl. II, Fig. 40), otherwise they almost hidden the inside of cells. Noteworthy were the pretty large pyrenoids (Pl. II, Figs 32, 34, 35, 39, 42) visibly differing from the previous cultures. The role played by O₂ in CO₂ assimilation has been a long-lasting dispute in plant physiology (KESSLER 1960; DEMIDOV *et al.* 1980). The present investigations could only show that the decrease in O₂ concentration in the environment of cultures does not alter considerably either the general aspect of the population or the growth of this *S. acutus*, strain Mono.

In the two cultures containing molasses in the medium with or without CO₂ supply, large singular cells were characteristically overwhelming; only here and there could be seen loosely adjoined four- and two-celled coenobia (Pl. II, Figs 43—48; Pl. III, Figs 49—54), or alternating four-celled ones (Pl. II, Fig. 43), as well as cells adjoined merely by papillae (dactylo-

coccoidal stage; Pl. III, Fig. 51). The shape of cells was mostly ellipsoidal or broadly-fusiform, especially in the culture bubbled without CO₂ in the air (variant (d)). The poles gradually narrowing, with well-developed papillae or, in the case of asymmetric cells, broadly-rounded. Another peculiarity recorded in this culture was the frequency of mother cells, or better said, the difficulty of autospore liberation, probably, induced by the resistance or mucilaginousness of cell walls (Pl. II, Figs 47-48; Pl. III, Fig. 54). Aberrant forms were not infrequent (Pl. II, Fig. 46). The chloroplasts were enlarged enough with sinuous margins and relatively big pyrenoids, but in parallel culture supplied with CO₂ they appeared perforated and tiny granulated, bearing smaller pyrenoids (Pl. III, Figs 50, 52). Otherwise, the most luxuriant growth has been recorded in this last culture, a result agreeing with the data obtained by VENKATARAMAN and BECKER (1985).

The presence of glucose in the medium resulted in an almost complete lack of coenobia, existing only small colonies of cells linked to each other by their poles (Pl. III, Figs 55-59), or loosely touched on sides (Pl. III, Figs 55, 57, 58).

The preponderance of monodesmoidic forms in cultures with molasses and glucose arises the question whether this appearance is a peculiarity of this algal strain or only a consequence of bubbling. Earlier observations (SENN 1899; GROSSMANN 1921) ascertained that glucose promoted the development of coenobia of closely attached cells (in static cultures, of course) in S. acutus and S. caudatus. Furthermore, it is asserted that "coenobium formation in S. obliquus (Indiana strain, similarly in static cultures) was favored by the addition of 1% glucose to the medium" (TRAINOR 1964), while S. intermedium (a strain from Peru) "needs an organic component for producing coenobia" (HEGEWALD et al. 1978). The "coenobiagram" was also sensitively modified in S. quadrispina; wheat extract promoting the ratio of scenedesmoidic coenobia (NAGY-TÓTH 1964). This peculiar aspect of the present S. acutus populations grown under the effects of molasses and glucose could be a consequence of the high variability of cell shapes, i.e. broadly-ellipsoidal (Pl. III, Figs 57-59), ellipsoidal (Pl. III, Fig. 56), asymmetrically elongated (Pl. III, Fig. 55), most of them having widely rounded poles and small and short apices. These sizes are close to those cells grown in media containing molasses, especially to those running without CO₂ supply. Characteristic was the aspect of chloroplasts covering almost entirely the inner part of the cell wall, but having perforations and granulations as well, while the pyrenoids were variable, mostly small.

Accumulation of starch in chloroplasts of S. acutus under the effect of sugars has already been mentioned even by BEIJERINCK (1890). On the other hand, in the present experimented strain Mono of S. acutus glucose did not destroy chloroplasts, did not break down chlorophyll, as this process has been reported in some Chlorococcacean algae (GROSSMANN 1921; MATSUKA, HASE 1965), since the cells remained nicely green and the culture grew vigorously. It might be worth mentioning here that especially glucose, but generally all cell-absorbed carbohydrates increase their sizes making them nearly spherical; swollen on the sides (fattening). This phenomenon can occur not only in Scenedesmus species, but also in other green algae (BEIJERINCK 1890; BERGMANN 1955; SENTSOVA et al. 1988), and perhaps in all living organisms. Thus, the well-known rod shaped cells of Stichococcus species have been transformed into spherical ones under the influence of tryptic soy broth and glucose, which according to SOROKIN and NISHINO (1973), in a consequence of the retention of growth. It is not sure that this explanation could be valid for the cultures of S. acutus. Mono, since it multiplied fast enough and its cells were even bigger than in control. Still, the fattening effect of carbohydrates reminds on KLUYVER's (cit. STANIER et al. 1973) "unity of biochemistry" rule.

The population grown in a solution supplied with ethanol but deprived of extra CO₂ resembled much that of an O₂-less culture, both in coenobia organization and in cell shape. Four-celled coenobia of loosely attached and slightly alternating cells were rather frequent (Pl. III, Figs 60--64) and singular cells were preponderant (Pl. III, Figs 62--63). The cells were mostly characteristically fusiform, with gradually narrowing poles and short papillae. The chloroplasts were enlarged, with lobated or fringed margins, always holding clearly visible pyrenoids. The other cultures with ethanol and supplied with CO₂ was dominated by singular cells, among which a few weakly aggregated colonies occurred (Pl. III, Figs 65--70). Interesting was the occurrence of aberrant lunate (crescent-shape) cells (Pl. III, Figs 69--70) having three poles and apexes each (Pl. III, Fig. 69). The shape of cells was rather variable; they were more or less fusiform and nearly cylindrical, with gradually narrowing or slightly bluntly ended poles and short papillae (Pl. III, Figs 65--66). Chloroplasts were smooth or granulated and perforated, holding small pyrenoids (Pl. III, Figs 66, 69).

Resuming these observations, it seems interesting to mention that the almost generally accepted enhancement effect of ethanol on the growth of Chlorococcacean algae (Scenedesmus species inclusively) (CONRAD, SALTMAN

1964) is probably performed without producing drastical changes in their morphological structure.

Electron microscopy of vegetative cells

The aim of these investigations was to detect any possible changes induced by various carbon sources and the absence of molecular oxygen in the air on the fine structure of cells of Scenedesmus acutus, strain "Mono", grown in batch cultures.

The main findings are illustrated in Plates IV--VIII.

The population grown solely on inorganic nutrients (Kuhl--Lorenzen's medium) and bubbled with a mixture of air and CO₂ (control), exhibited normally looking monodesmoid coenobia, having ellipsoidal to broadly-ellipsoidal cells. The cell wall was relatively thin, with wavy outline, having the usual structure. The cells exhibited the expected inner organization of green alga type (Pl. IV, Figs 71, 72), with massive parietal chloroplast containing a large pyrenoid and a relatively large nucleus. The chloroplast had 5-10 lamellae running \pm parallel with the cell surface, each consisting of 3-5 closely stacked thylakoids. The sections (Pl. IV, Figs 71, 72) revealed a reduced number of intraplastidial starch grains. The pyrenoids were relatively large, being surrounded by double starch sheath. The cytoplasm, distributed mainly in the internal cavity of the chloroplast, contained the usual structural element: a large nucleus with a single nucleolus, a few perinuclear Golgi profiles, several small mitochondrial profiles located near the inner surface of the chloroplast, as well as a few ER profiles connected with the nuclear envelope (Pl. IV, Fig. 72). The ground cytoplasm was very rich in ribosomes, which were also seen on the surface of the nuclear envelope and ER cisternae. The periplastidial cytoplasm layer, equally rich in ribosomes, exhibited many small mitochondrial profiles (Pl. IV, Fig. 72).

Cells grown in the absence of supplementary CO₂ and without O₂ (Pl. V, Figs 73, 74) showed a somewhat different structure. They were more elongate and attenuated towards their ends, some of them having irregular outline. The bulging pyrenoids were markedly larger, with a seemingly continuous starch sheath; the chloroplasts have very few and small starch grains (these were mostly absent). The number of mitochondrial profiles was much reduced, but they were more elongated, sometimes branched. The chloroplast lamellae were more regularly spaced as in the control.

The structure of cells grown in the presence of excess CO_2 , but lacking molecular oxygen were largely the same, at first sight, as that of the control. It was interesting to observe that the chloroplast lamellae were fewer and the pyrenosomes were somewhat larger, and surrounded by two starch cups. The cell wall was mostly doubled (Pl. V, Figs 75, 76; Pl. VI, Fig. 77), less wavy, almost even. Some of the cells were overloaded with starch grains.

In the cultures grown in the presence of molasses, with or without CO_2 supply, all cells were overloaded with starch grains (Pl. VI, Figs 78, 79), the massive and shapeless chloroplast filling almost entirely the cell cavity. The cytoplasm was restricted to a small central space containing a small nucleus and a few of the other usual structural elements. The net-like chloroplast lamellae have been surrounding large, irregularly distributed starch grains; pyrenoid mostly not seen or with very reduced pyrenosome when supplied with CO_2 . The thylakoids were often swollen and partly disintegrated. The cell wall were very thick and doubled, lacking the wavy outline.

The presence of glucose induced a few differences (Pl. VII, Figs 80, 81); the cell wall was almost smooth, thick, its inner layer being very well developed. The chloroplast lamellae were similar with those of the control, but with many large starch grains. The cytoplasm contained the usual structural elements and many large, roughly globular inclusions of unknown nature. Such inclusions, strongly electron-dense, were also present in most cells of the control (Pl. IV, Figs 71, 72) and of other variants too (Pl. V, Figs 75, 76).

The cultures grown in the presence of ethanol and bubbled with air only were composed of cells with thinner cell wall and fine, delicate chloroplast, having but a few small, intraplastidial starch grains (Pl. VII, Fig. 82). The thylakoids were irregularly grouped and often swollen; single thylakoid membranes were present in the matrix. The outline of the chloroplast was not clear-cut as usual. The pyrenosome was partly penetrated by thylakoid cisternae. Mitochondrial profiles were few, their fine structure being highly damaged showing vesicles instead of characteristic cristae (Pl. VII, Fig. 82, double arrow). Many inclusions could be seen in the cytoplasm; they were of various size and structure (granular, membranous, alveolar) some of them seemingly were degenerated cell organelles (mitochondria).

When the ethanol supplied cultures were supplemented with CO_2 too, then the structure of cells resembled that of the control (Pl. VIII, Figs 83, 84). The cell wall was more or less wavy in outline, not very thick, sometimes doubled. The cytoplasm contained many inclusions, partly or entirely dissolved by fixatives. The most striking difference between the structure of such cells, when compared with the control, concerns the periplastidial cytoplasm containing not only the usual mitochondria, but also large, presumably oil droplets (Pl. VIII, Fig. 83, double arrow).

Conclusions

The structure of coenobia and the cell shape in Scenedesmus acutus Meyen, strain Mono were sensitively influenced by cultivation conditions, i.e. static or batch (bubbled) cultures, composition of nutrient media, carbon sources.

In Kuhl--Lorenzen's nutrient solution in static cultures (subcultures), this alga occurred as four-, two- and one-celled coenobia; in four-celled coenobia the cells were frequently slightly alternating, while in batch cultures the population consisted overwhelmingly of singular cells, which, however, exhibited an electronmicroscopical structure near to those grown in natural conditions. In both these cultures the shape of cells was highly variable. Similarly in batch cultures in Knop--Pringsheim--Felföldy's and Tamiya's solutions singular cells were dominant, but four and two-celled coenobia were also frequent.

In batch cultures grown without O_2 in the bubbling air the ratio of four-celled coenobia was higher and the variability of cell shape lower. The cells, seemingly with normal structure, developed larger pyrenoids.

Molasses, glucose and ethanol added to the Kuhl--Lorenzen's nutrient medium did not bring about chloroplast destruction, however damaged it profoundly, the cultures did not turn yellow, which could denote the genetically strongly fixed autotrophy (mixotrophy) of this alga. Molasses and glucose (and the soil extract existing in Knop--Pringsheim--Felföldy's solution) led to an increase the cell size. The strongest effect was exerted by molasses, especially in the culture supplied with extra amount of CO_2 . In weakened coenobia formation, but did not annihilate the four-celled coenobial stage. The cells were overloaded with starch grains and the chloroplasts were highly denaturated. In glucose-containing cultures four- and two-celled coenobia could be seen only incidentally; the cell wall was thicker.

The effect of ethanol was evident especially when CO₂-supply was omitted. The mitochondrial profiles exhibited internal vesicles instead of cristae, the thylakoids were often swollen and isolated.

REFERENCES

- Artar, A. (1899): Über die Entwicklung der grünen Algen unter Ausschluss der Bedingungen der Kohlensäure-Assimilation. Bull. Soc. Imp. Natur. Moscou, Nouv. Série 13 (1): 39–47.
- Becker, E. W. (1982): Ernährung, Wachstumsleistung, Ertrag und Konkurrenzfähigkeit von Algenmassenkulturen. Kali-Briefe (Büthenhof) 16 (5): 249–269.
- Beijerinck, M. W. (1890): Kulturversuche mit Zoochlorellen, Lichenengonidien und anderen niederen Algen. Bot. Ztg. 48: 725–785.
- Bergmann, L. (1955): Stoffwechsel und Mineralzernährung einzelliger Grünalgen II. Vergleichende Untersuchungen über den Einfluss mineralischer Faktoren bei heterotropher und mixotropher Ernährung. Flora (Jena) 142: 493–539.
- Blazhenova, A. N., Ilinskaya, A. A., Rapoport, F. M. (1957): Analiza gazelor în Industria Chimică. Ed. Technică, București, pp. 68–71.
- Chambers, C. O. (1912): The relation of algae to dissolved oxygen and carbon-dioxide. With special reference to carbonates. Twenty-third Ann. Mo. Bot. Gard. XXIII: 171–207.
- Conrad, H. M., Saltman, P. (1964): Growth substances. In: Lewin, R. A. (ed.): Physiology and Biochemistry of Algae. Acad. Press, N. Y., London, pp. 663–671.
- Demidov, E. D., Kerimov, S. Kh., Borodin, V. B., Bell, L. N. (1980): Fotosintez i fotodykhanie kletok khlorelly, adaptirovannykh k nizkim i vysokim kontsentratsiyam uglekisloty. Fiziol. Rast. 27: 1018–1023.
- Gavis, J., Chamberlin, C., Lystad, L. D. (1979): Coenobial cell number in Scenedesmus quadricauda (Chlorophyceae) as a function of growth rate in nitrate-limited chemostats. J. Phycol. 15: 273–275.
- Goldman, J. C., Porcella, D. B., Middlebrooks, E. J., Toerien, D. F. (1972): The effect of carbon on algal growth. Its relationship to eutrophication. Water Res. 6: 637–679.
- Grintzesco, J. (1902): Recherches expérimentales sur la morphologie et la physiologie de Scenedesmus acutus Meyen. Bull. Herb. Boissier Sec. Série 2 (3): 217–288.
- Grossmann, E. (1921): Zellvermehrung und Koloniebildung bei einigen Scenedesmaceen. Int. Rev. Ges. Hydrobiol. Hydrograph. 9: 1–58.
- Hegewald, E., Aldave, A., Schnepf, E. (1978): Investigations on the lakes of Peru and their phytoplankton. 2. The algae of pond La Laguna, Huanuco, with special reference to Scenedesmus intermedius and S. armatus. Arch. Hydrobiol. 82: 207–215.
- Hellebust, J. A., Yu-Hung Lin (1978): Uptake of organic substrates. In: Hellebust, J. A., Craigie, J. S. (eds): Handbook of Phycological Methods. Cambridge Univ. Press, pp. 379–388.
- Hortobágyi, T. (1941): Újabb adatok a Tisza Nagyfa-holtága fitoplanktonjának kvalitatív vizsgálatához. I. Bot. Közlem. 37 (3–4): 150–170.
- Hortobágyi, T. (1957): Algák két hortobágyi halastóból. Egri Ped. Főisk. Fü. 74: 1–48.
- Hortobágyi, T. (1978): The Microflora in the Settling and Subsoil Water Enriching Basins of the Budapest Waterworks. Akad. Kiadó, Budapest, 84 pp.

- Kessler, E. (1960): Der Einfluss von Sauerstoff auf die Photosynthese. In: Ruhland, W. (Hrsg.): Handbuch der Pflanzenphysiologie 5/1, Teil 1, Springer Verl., Berlin, pp. 935—950.
- Matsuka, M., Hase, E. (1965): Metabolism of glucose in the process of "glucose-bleaching" of Chlorella protothecoides. Plant and Cell Physiol. 6: 721—741.
- Nagy-Tóth, F. (1964): Contribuții la cunoașterea variației talurilor de Scenedesmus quadricauda Chod. sub influența substanțelor stimulative de creștere. Contrib. Bot. (Cluj) pp. 321—328.
- Nagy-Tóth, F. (1972): Cercetări privitoare la cultivarea intensivă a unor alge verzi. Teza Dr. Univ. "Babeș-Bolyai", Cluj, pp. 1—333.
- Nagy-Tóth, F. (1987): Notes on the pleomorphism of Scenedesmus intermedius Chod. Arch. Hydrobiol. Suppl. 68: 325—342.
- Overbeck, J., Stange-Bursche, M.-E. (1966): Experimentelle Untersuchungen zum Coenobiumformwechsel von Scenedesmus quadricauda (Turp.) Bréb. Ber. Deutsch. Bot. Ges. 78 (9): 357—372.
- Péterfi, L. S., Dragos, N., Craciun, C. (1979): Ultrastructure of the schizogoniaceous green alga Klebsormidium pseudostichococcus (Heering) comb. nova: organization of vegetative cells. Rev. Roum. Biol., Ser. Biol. Veget. 24 (1): 77—80.
- Péterfi, L. S., Momeu, L., Nagy-Tóth, F., Craciun, V. (1988): Observations on the fine structure of Chlorococcum minutum Starr (Chlorococcales, Chlorophyceae). Vegetative cell and zoospores. Arch. Protistenk. 135: 133—145.
- Senn, G. (1899): Über einige coloniebildende einzellige Algen. Inaugural-Dissertation der Universität Basel. Basel, pp. 2—70.
- Sentsova, O. Yu., Kravtsova, T. R., Gusev, M. V. (1988): Physiological and biochemical properties of the acidothermophilic algae Cyanidium caldarium and Galdieria sulfuraria and their growth in mixed cultures under photoautotrophic, mixotrophic and heterotrophic conditions. Fiziol. Rast. 35 (3): 516—525.
- Sorokin, C., Nishino, S. (1973): Transformation of rod-shaped algal cells into spherical cells. Amer. J. Bot. 60: 907—914.
- Stanier, R. Y., Doudoroff, M., Adelberg, E. A. (1973): General Microbiology. MacMillan Press Ltd., London, pp. 559—576.
- Steenbergen, C. L. M. (1978): Pleomorphism of Scenedesmus quadricauda (Turp.) Bréb. (Chlorophyceae) in synchronized cultures. Mitt. Int. Limnol. 21: 216—223.
- Trainor, F. R. (1964): The effect of composition of the medium on morphology in Scenedesmus obliquus. Canad. J. Bot. 42: 515—518.
- Trainor, F. R., Cain, J. R., Shubert, L. E. (1976): Morphology and nutrition of the colonial green alga Scenedesmus: 80 years later. Bot. Rev. 42 (1): 5—25.
- Uherkovich, G. (1966): Die Scenedesmus-Arten Ungarns. Akad. Kiadó, Budapest, 39 pp.
- Venkataraman, L. V., Becker, E. W. (1985): Biotechnology and Utilization of Algae. — The Indian Experience. Publ. Dept. Sci. and Technol., New Delhi, pp. 1—67.
- Weier, E. T., Stocking, C. R., Barbour, M. G. (1970): Botany. An Introduction to Plant Biology. J. Wiley and Sons, N. Y., pp. 441—479.

Plate I

Figs 1—4. Subculture (static) grown in Kuhl—Lorenzen's solution

Figs 5—15. Batch culture in Kuhl—Lorenzen's solution

Figs 16—24. Batch culture in Knop—Pringsheim—Felföldy's solution

Figs 25—31. Batch culture in Tamiya's solution

Plate II

Figs 32—37. Culture bubbled without O₂ in the air and without CO₂

Figs 38—42. Air without O₂, but supplied with CO₂ (1.5–3%)

Figs 43—48. Culture bubbled without CO₂, but supplied with molasses

Plate III

Figs 49—54. Culture supplied with molasses and CO₂

Figs 55—59. Culture supplied with glucose

Figs 60—64. Culture supplied with ethanol

Figs 65—70. Culture supplied with ethanol and CO₂

Plate IV

Figs 71—72. Electron microscopy of *Scenedesmus acutus* Meyen grown in batch culture in Kuhl—Lorenzen's solution (control); 71. — Oblique sections cut through vegetative cells at different levels, showing the cell wall (CW), chloroplast (CHL), cytoplasm (cy) with Golgi body (arrow) and mitochondrial profiles (arrow head), nucleus (N) and nucleolus (n) (16000 x)

Plate V

Figs 73—76. Batch culture grown without O₂ in the bubbling air, in Kuhl—Lorenzen's solution

73—74. — Oblique section of vegetative cells, showing the cell wall (CW), chloroplast (CHL), pyrenoid (Py) and cytoplasm (cy) (8000 x)

75—76. — Part of oblique sections cut through vegetative cells from the culture grown without O₂, but supplied with CO₂, Kuhl—Lorenzen's solution; the layered cell wall (WV), cytoplasm (cy) with mitochondrial profiles (m), chloroplast (CHL) containing a pyrenoid (Py) and starch grains (S); the nuclear envelope (Nm) surrounded nucleus (N) contains a single nucleolus (n) (28000 x)

Plate VI

Figs 77—79. Batch culture in Kuhl—Lorenzen's solution bubbled without O₂, but supplied with CO₂; note the organization of pyrenoid (Py) and cell wall (CW) (20000 x)

78. — Batch culture in Kuhl—Lorenzen's solution supplemented with molasses and bubbled with air supplied with CO₂; cell wall (CW), chloroplast (CHL) overloaded with starch grains (S) (20000 x)

79. — Oblique section of a cell from the same culture; the chloroplasts overloaded with starch (8000 x)

Plate VII

Figs 80—82. Batch culture grown in presence of glucose

80. — Oblique section of a cell, showing the structure of cell (CW), chloroplast (CHL) with starch grains, cytoplasm with inclusions of unknown nature (arrow) and nucleus (N) (20000 x)

81. — Part of oblique section presenting the cell wall and chloroplast structure (48000 x)

82. — The effect of ethanol on the structure of chloroplast (CHL), pyrenoid (Py) which is partly penetrated with thylakoids, cytoplasm with Golgi profiles (arrow), denaturated mitochondrial profiles (double arrow), as well as many inclusions (I) of unknown nature (20000 x)

Plate VIII

Figs 83—84. Oblique sections of cells grown in presence of ethanol. The sections reveal the structure of cell wall (CW), starch-loaded (S) chloroplast (CHL), cytoplasm (cy) with Golgi (arrow heads), mitochondrial profiles (arrow) and partly dissolved cytoplasmic inclusions of unknown nature (I), as well as oil droplets (double arrows) (20000 x)

Plate I

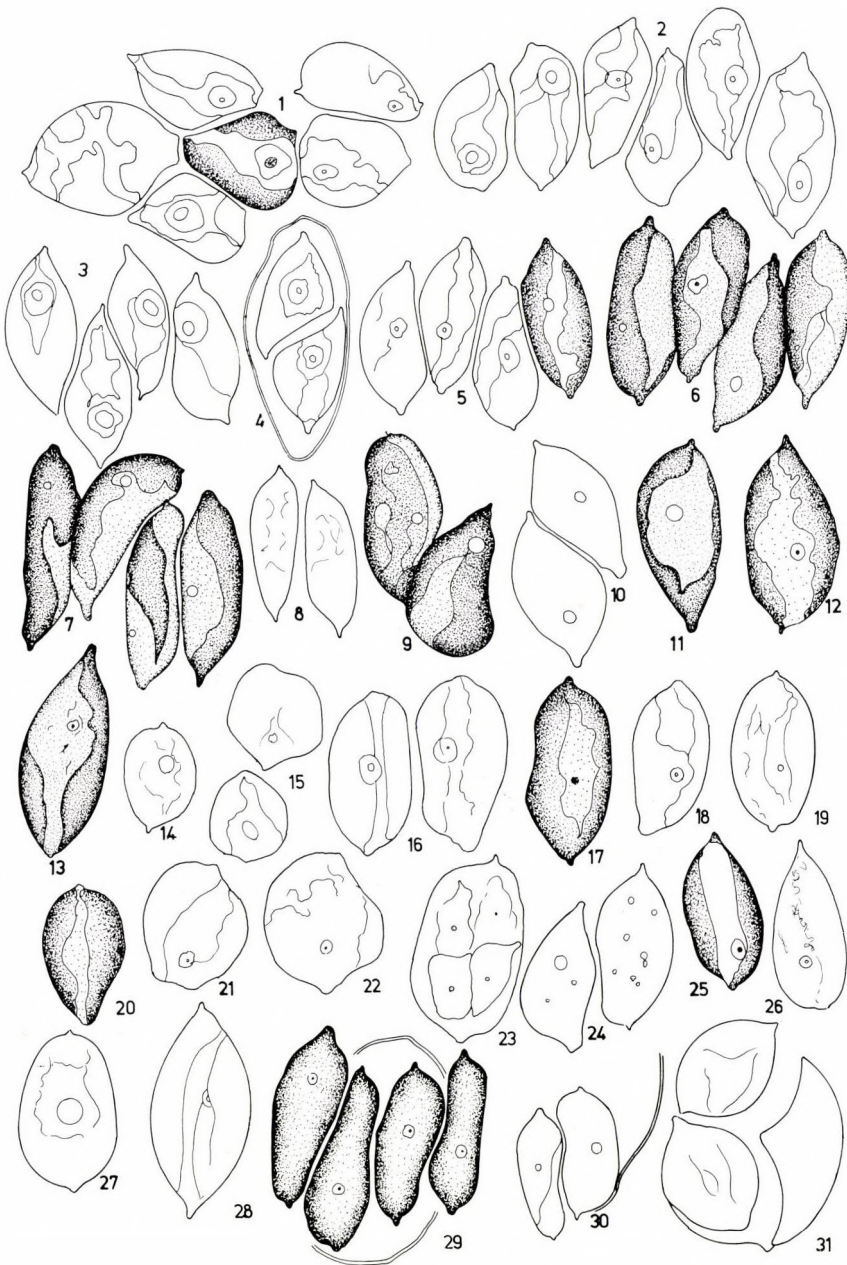


Plate II

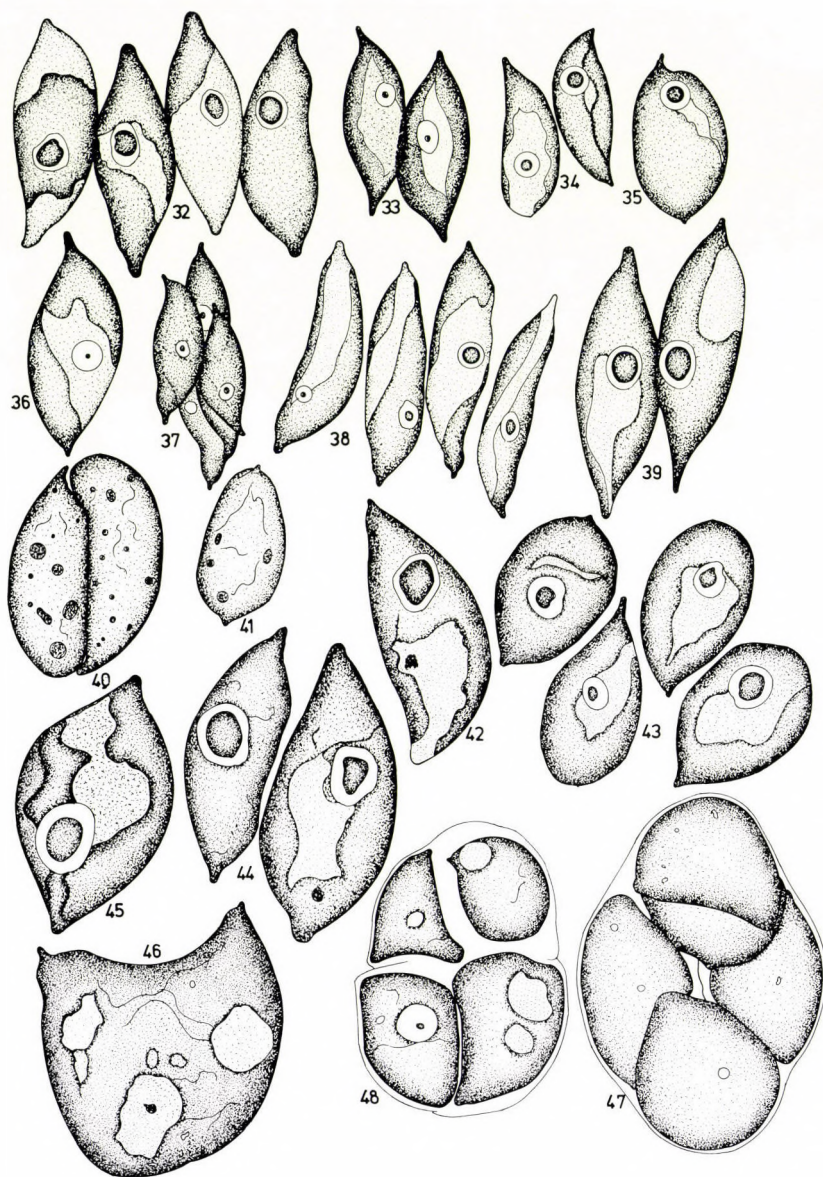


Plate III

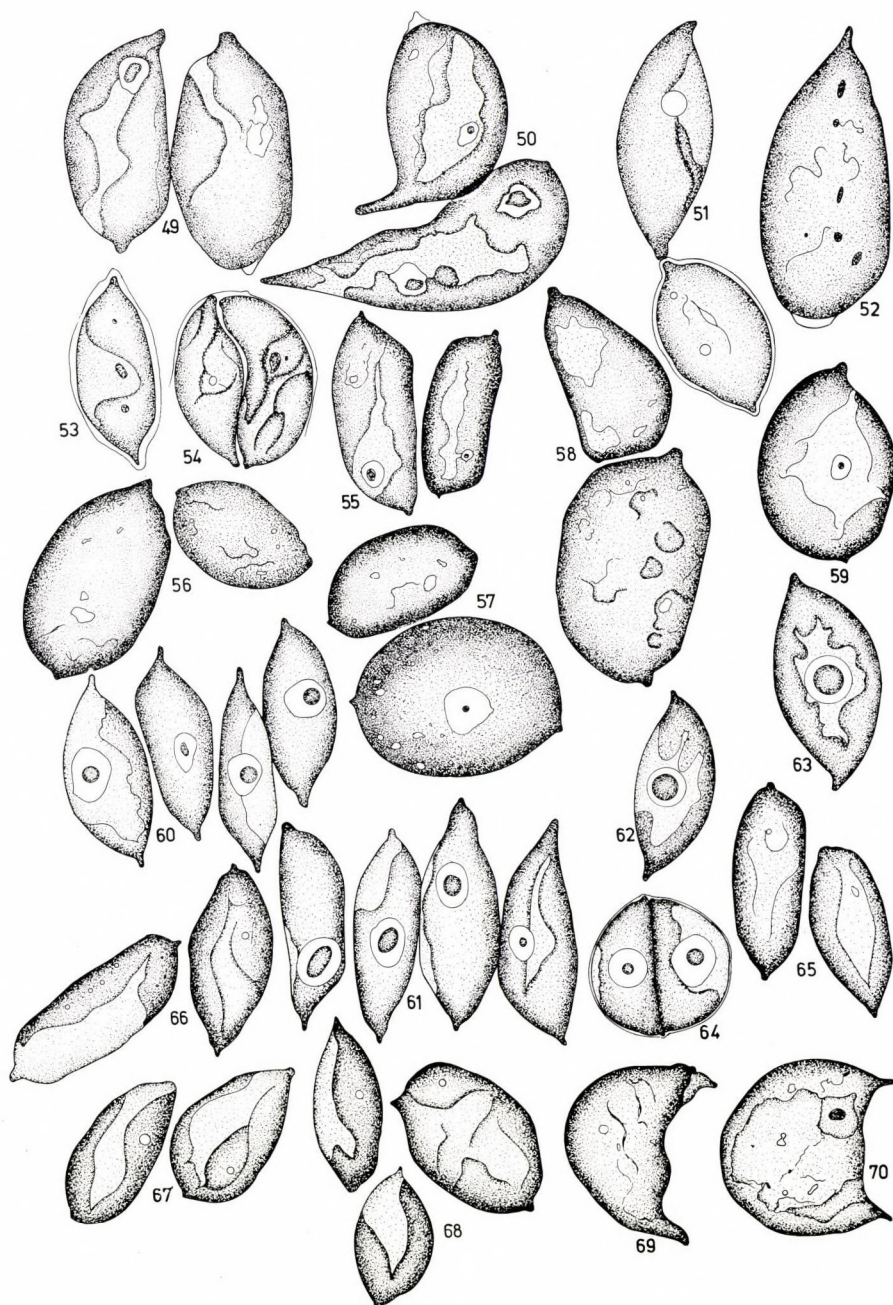


Plate IV

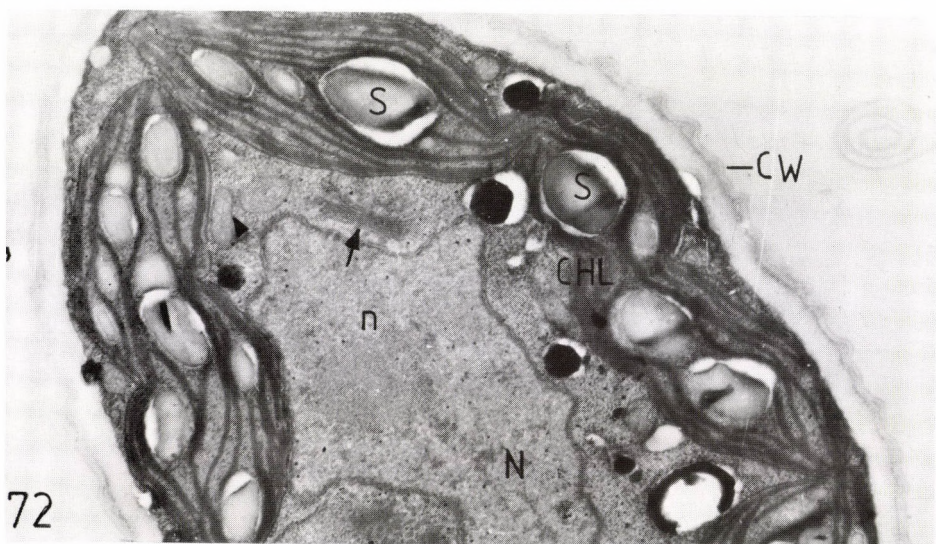
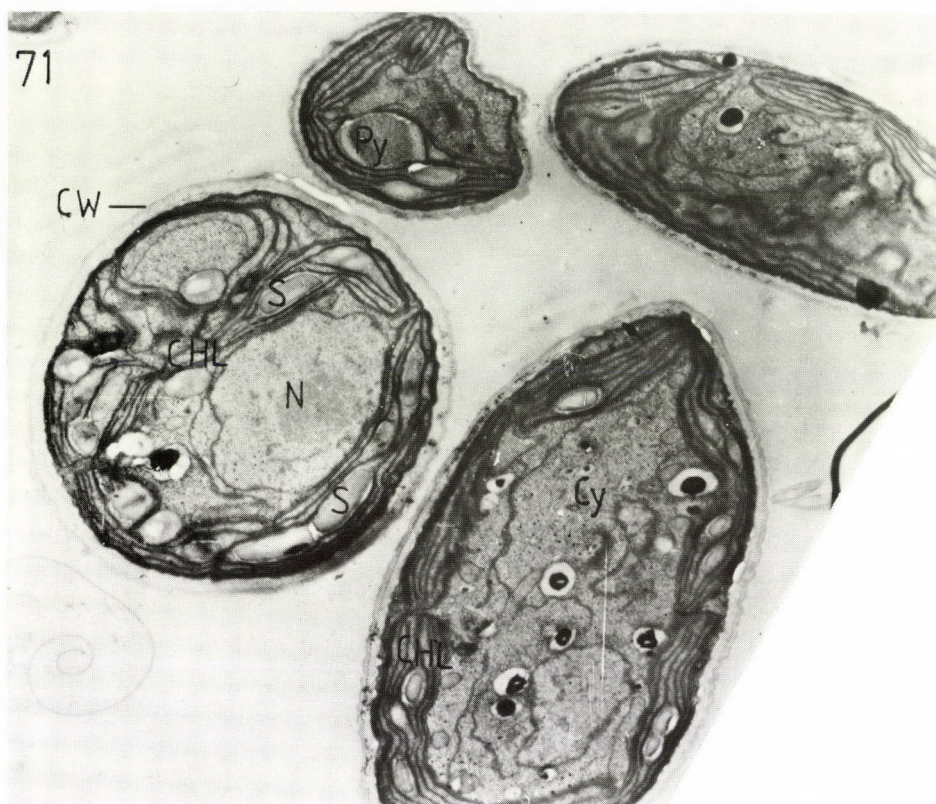


Plate V

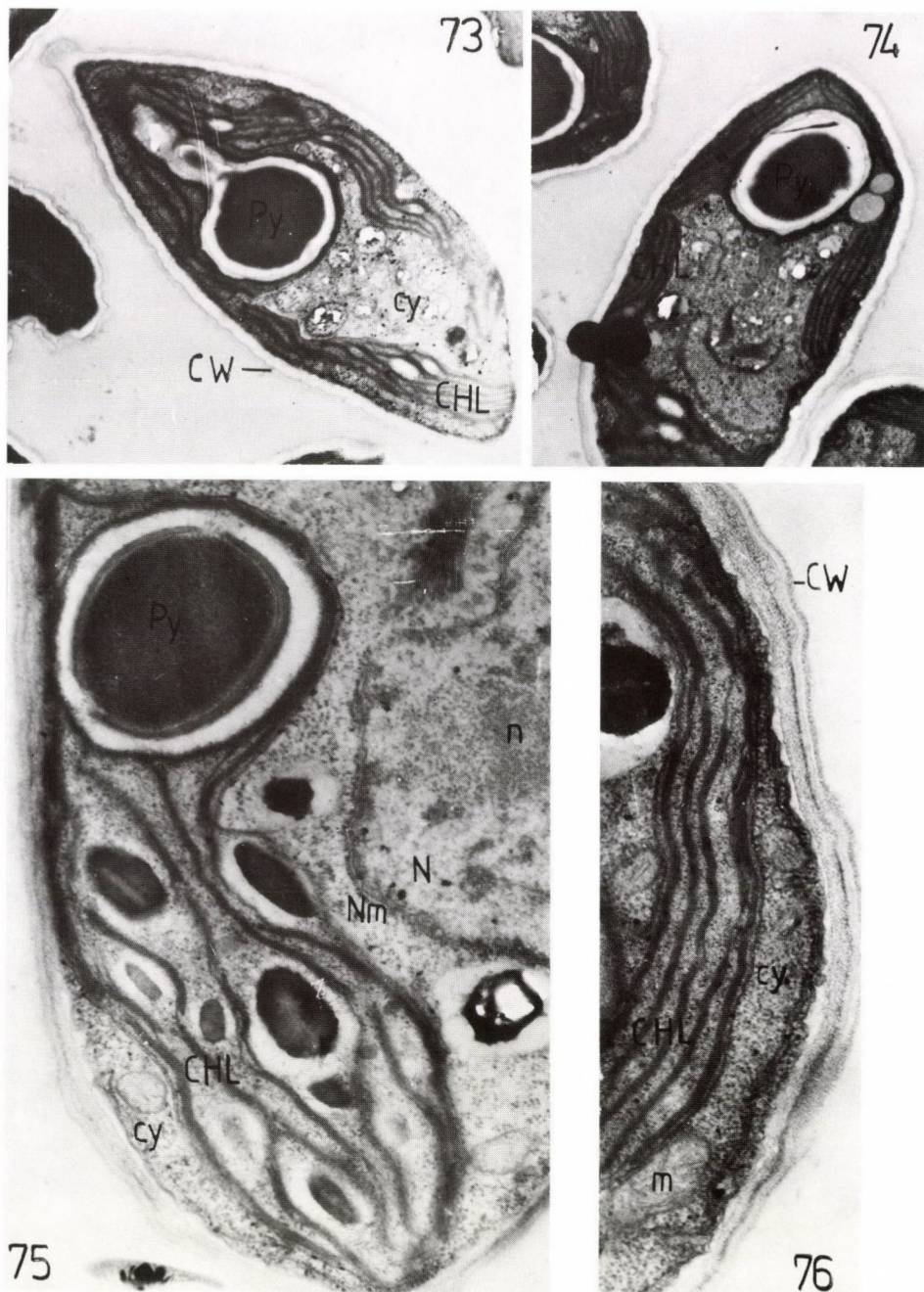


Plate VI

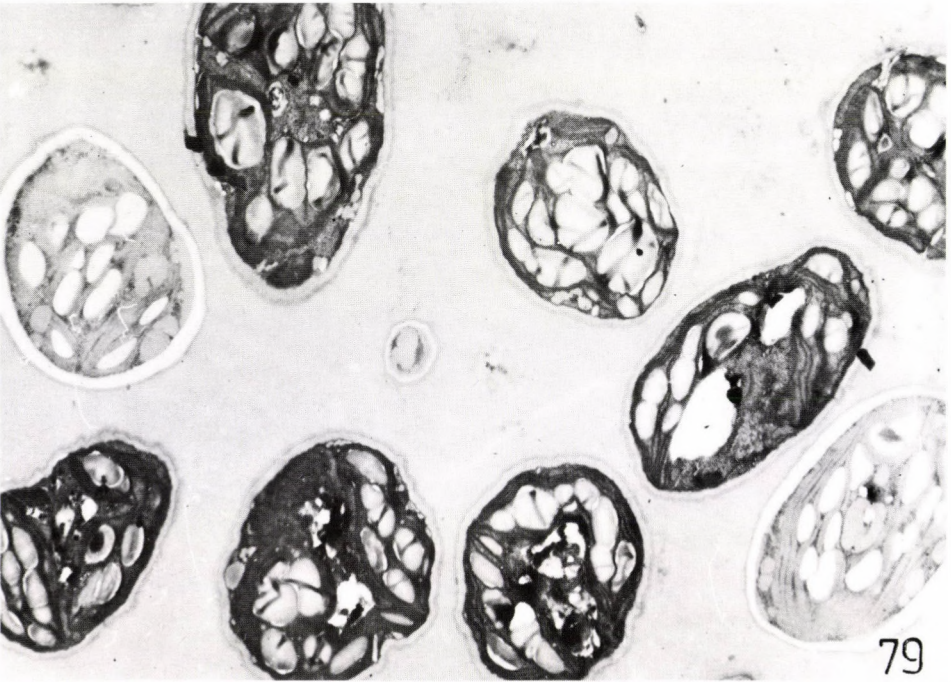
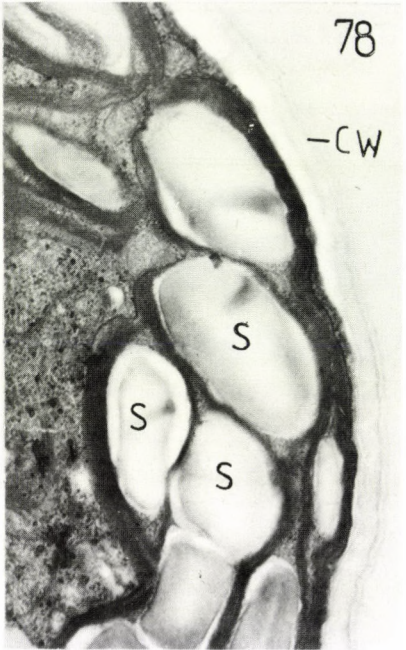
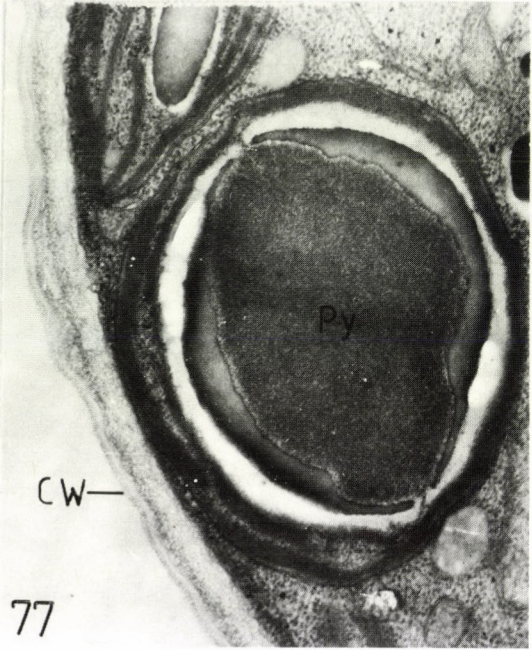


Plate VII

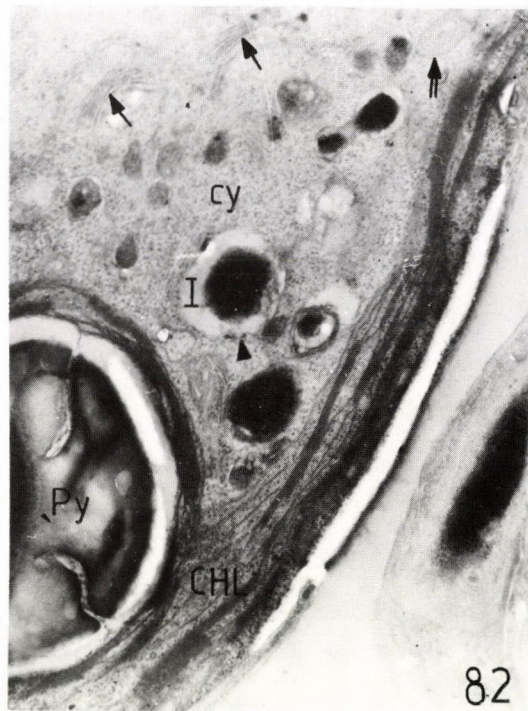
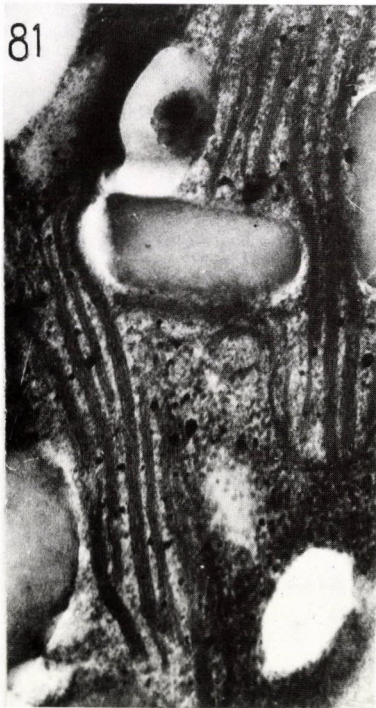
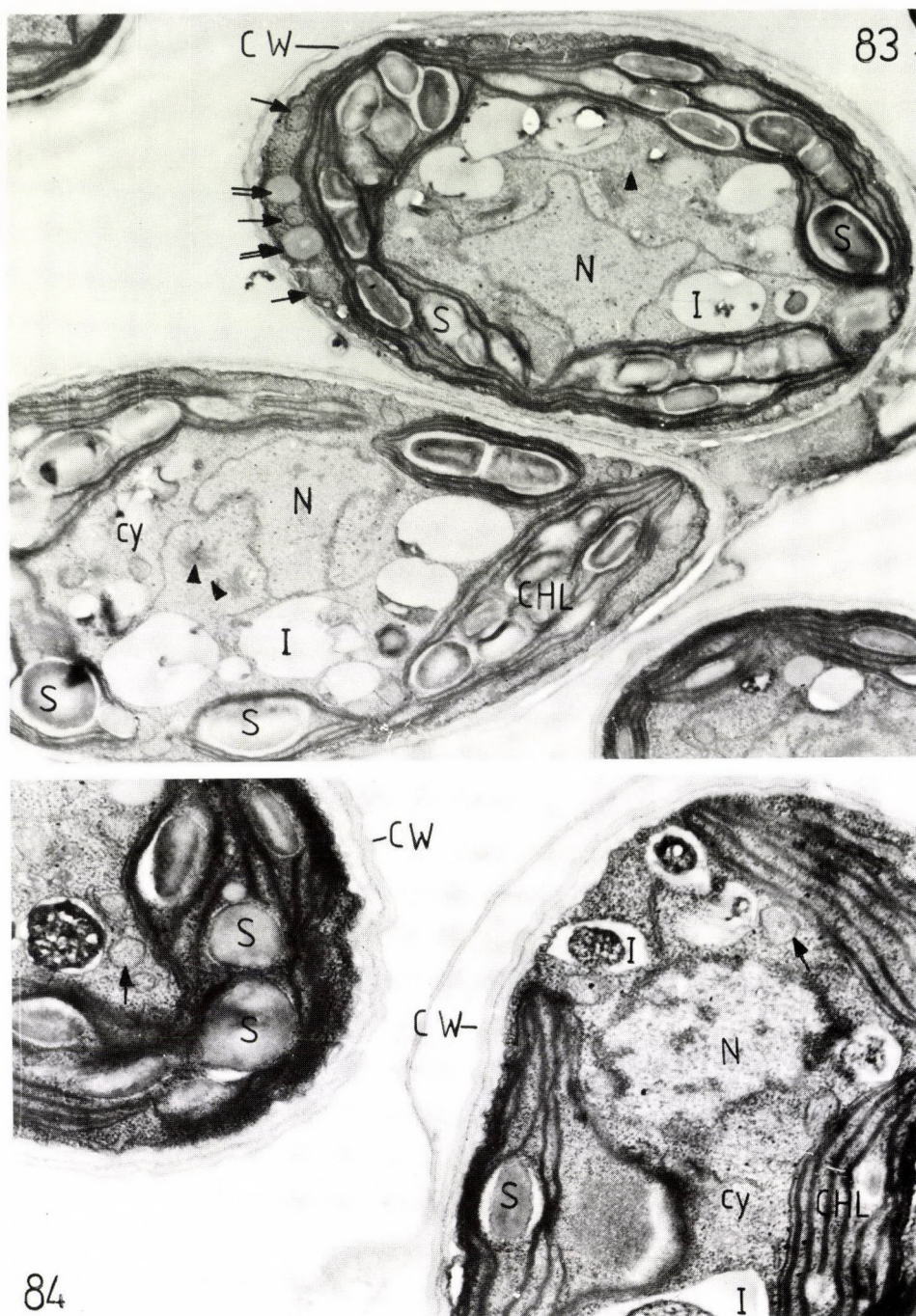


Plate VIII



DETERMINATION OF RADIOACTIVITIES IN SOME SPECIES OF HIGHER FUNGI

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The radioactivities of 26 samples of mushrooms (22 species) were determined. These samples originated primarily from the parkforest near Pestszentlőrinc (Hungary). Samples were taken from different parts of the Pestszentlőrinc forest including samples from pine, oak and acacia. Measurements included isotopes such as K-40, Cs-134, Cs-137, Ag-110 m, total-beta and Sr-90. It was found the uptake of cesium isotopes in mushrooms is higher than the others green plants, and Macrolepiota species are especially suitable test organism to detect Ag-110 m isotope.

Introduction

It was reported earlier in 1960-1970 that some of the higher fungi are able to bioaccumulate Cs-137 (GRUETER 1971; HASELWANDTER 1978). The number of such publications increased significantly after the explosion of the nuclear power plant in Chernobyl. Authors registered high activities of cesium isotope in mushrooms even in places which were far away from the catastrophe. The most activities of cesium isotopes they could measured for example: in Paxillus involutus, Laccaria laccata, L. amethystina, Cortinarius armillatus species and in Xerocomus genus (HORYNA and RANDA 1988; GANS 1987; MOLZAHN et al. 1989; RANDA 1988; TEHERANI 1987; HASELWANDTER 1978; DIETL 1989; RÜCKERT and DIEHL 1987). Potassium is known to form a coloured complex named norbadion A found in the skin of mushroom Xerocomus badius which can be replaced by cesium (STEFFAN and STEGLICH 1984). This would explain the increased uptake of cesium isotopes in Xerocomus badius.

Based on such background we have started to collect mushroom samples at the Pestszentlőrinc parkforest, near Budapest and have analysed their radionuclides content. In this paper we describe a high uptake of cesium isotopes in different mushroom species. The collected and measured samples are in Eumycota division in Ascomycotina and Basidiomycotina subdivision.

Material and Methods

Freshly collected mushrooms were dried at 105 °C and then burned to ashes at 350 °C. The ashes of these samples were used first for gamma-spectrometry then for the measurement of total-beta radioactivity, finally followed by counting separately the Sr-90 content. The soil samples came from growing areas of mushroom mycelia collected together with forest litter from the top 5 cm layer of the soil. Soil samples were dried at 105 °C until their weight did not change further then were homogenised, sifted on 21 mesh sieve and finally gamma-spectrometric measurements were carried out.

Gamma-spectrometry

The radionuclide analysis of samples was performed by gamma-spectrometry. Gamma spectra were recorded on a HPGe detector of 30.9% relative efficiency and 1.92 keV resolution and peak Compton ratio: 1:45 with respect to the 1332.5 keV energy of the Co-60 isotope. The natural radioactivity background was lowered by a triple layer shielding consisting of lead cadmium and copper electrolytes.

Total-beta activity

For the measurement of the total-beta activity the detecting system consisted of an ND-350/F NaJ(Tl) single crystal, detector constructed from thin plastic scintillator surrounded by 5 cm thick lead protection and an NK-350 amplitudoanalyzer.

Determination of Sr-90 radioactivity

The Sr-90 isotope and its daughter element Y-90 are pure beta-emitters, thus the measurements were preceded by their radiochemical separation. Twelve days were given to reach equilibrium between the Sr-90 parent and Y-90 daughter elements (BENES and KYRS 1986; MONTAG 1965). For measurements the system described under total-beta activity was applied.

Result and Discussion

The activities of K-40, Cs-134, Cs-137 and Ag-110m were related to the dry weight of mushroom samples are summarized in Table 1.

The relationship between the mushroom/soil and that of isotope concentrations of K-40 and Cs-137 was calculated from data of Table 1 are compiled in Table 2. The total-beta and the Sr-90 + Y-90 measurements reflecting the activities of ashes with respect to their dry weight are seen in Table 3.

K-40 activities of samples

The average concentration of K-40 activities was 1285 Bq kg⁻¹ d.w., the highest amongst the nuclides tested, in accordance with the finding that the natural potassium content of mushrooms is high. The transferfactors of mushrooms versus soil fell between values 2.6 and 16.1 with an average of

Table 1

Radioactivity values of ^{40}K , ^{134}Cs , ^{137}Cs , $^{110\text{m}}\text{Ag}$ isotopes of higher fungi

Name	^{40}K error		^{134}Cs error		^{137}Cs error		$^{110\text{m}}\text{Ag}$ error	
	Bq kg ⁻¹ d.w.	%	Bq kg ⁻¹ d.w.	%	Bq kg ⁻¹ d.w.	%	Bq kg ⁻¹ d.w.	%
<i>Morchella esculenta</i>	948	20.5	-		3.2	0.41	-	
<i>Helvella lacunosa</i>	956	12.3	-		29.3	3.27	-	
<i>Paxina leucomelas</i>	1285	17.1	15.8	3.33	120.5	8.23	-	
<i>Laetiporus sulphureus</i>	1410	21.2	-		24.6	2.71	-	
<i>Suillus granulatus</i> '89	847	6.7	53.9	5.32	377.1	10.62	-	
<i>Suillus granulatus</i> '90	1107	17.7	77.5	2.71	697.5	10.18	-	
<i>Omphalotus olearius</i>	704	16.5	0.8	0.44	3.6	0.66	-	
<i>Tricholoma terreum</i> '89	1188	7.3	3.8	0.71	68.9	1.73	-	
<i>Tricholoma terreum</i> '90	2234	20.7	88.7	7.97	714	9.90	-	
<i>Tricholoma portentosum</i>	1730	5.8	43.6	8.21	252.1	3.21	-	
<i>Lepista nuda</i>	2098	16.5	42.0	3.28	257.5	5.67	-	
<i>Lepista sordida</i>	1266	25.3	-		127	2.34	2.2	0.31
<i>Lepista inversa</i>	1710	19.9	4.9	0.72	39.2	1.84	-	
<i>Lepista gilva</i>	1253	12.5	13.1	0.65	87.9	3.23	-	
<i>Armillariella mellea</i>	1630	19.7	1.6	0.26	17.4	1.11	-	
<i>Collybia dryophila</i> '89	1077	7.5	18.0	0.91	116.8	2.77	14.0	1.72
<i>Collybia dryophila</i> '90	811	19.5	37.7	1.42	269.9	4.01	-	
<i>Collybia butyracea</i>	1404	25.6	3.2	1.16	42.8	5.21	-	
<i>Marasmius oreades</i>	706	18.5	10.3	0.65	65.4	1.42	-	
<i>Entoloma clypeatum</i>	1272	13.3	17.1	2.14	86.3	5.91	-	
<i>Amanita phalloides</i> '89	1167	21.7	-		3.2	0.9	-	
<i>Amanita phalloides</i> '90	1612	187	-		11.3	3.00	-	
<i>Agaricus arvensis</i>	1392	26.0	3.6	0.71	22.7	2.84	-	
<i>Macrolepiota procera</i>	1496	29.3	-		18.0	0.90	5.2	0.91
<i>Macrolepiota rhacodes</i>	1183	31.7	-		24.2	1.22	1.5	0.57
<i>Lactarius vellereus</i>	930	18.0	8.9	0.45	108.3	2.01	-	

8.3 consequently these species are able to bioaccumulate of K-40 isotope. This finding corresponds to earlier observations with respect to the bioaccumulation of potassium (VETTER 1987).

Activities of Cs-137 of samples

The Cs-137 activities of soil samples collected at Pestszentlőrinc were surprisingly high. The activities of soils taken from forests were as follows: pine 715 Bq kg⁻¹ d.w. 1989, 248 Bq kg⁻¹ d.w. 1990, and oak 211 Bq kg⁻¹

Table 2

K-40 and cesium isotopes ratios in mushrooms and corresponding soils

Name	^{40}K Bq kg^{-1} d.w. mushroom	^{40}K Bq kg^{-1} d.w. soil	factor	Cs-isot. Bq kg^{-1} d.w. mushroom	Cs-isot. Bq kg^{-1} d.w. soil	factor
<i>Paxina leucomelas</i>	1285	130	9.9	136.3	715	0.19
<i>Suillus granulatus</i>	847	130	6.5	431	715	0.60
<i>Tricholoma terreum</i> '89	1188	130	9.1	72.7	715	0.10
<i>Tricholoma terreum</i> '90	2234	261	8.6	802.7	280	2.87
<i>Tricholoma portentosum</i>	1730	130	13.3	295.7	715	0.41
<i>Lepista nuda</i>	2098	130	16.1	299.5	715	0.42
<i>Lepista sordida</i>	1266	270	4.7	127	56.7	2.24
<i>Lepista inversa</i>	1710	270	6.3	44.1	56.7	0.78
<i>Lepista gilva</i>	1253	270	4.6	101	56.7	1.78
<i>Collybia dryophila</i> '89	1077	130	9.1	134.8	715	0.19
<i>Collybia butyracea</i>	1404	295	3.7	46	211	0.22
<i>Amanita phalloides</i> '90	1612	295	5.5	11.3	211	0.05
<i>Agaricus arvensis</i>	1392	130	10.7	26.3	715	0.04
<i>Macrolepiota rhacodes</i>	1183	270	4.4	18.0	56.7	0.31
<i>Collybia dryophila</i> '90	811	261	3.1	307.6	280	1.10

d.w. 1990, several times higher than the national average. Soil samples from acacia forest closely resembled those of the average Hungarian soil samples (56.7 Bq kg^{-1} d.w.) (RMS 1989; RMS 1990).

The average Cs-137 activity of mushroom samples was 155.1 Bq kg^{-1} d.w. between 3.2 - 802.7 Bq kg^{-1} d.w. within a range of two order of magnitude. The average mushroom/soil ratio of Cs-137 was 0.75, with a minimal 0.04 and a maximal 2.88 ratio.

Contrary to potassium the uptake of cesium showed a diverse pattern among different mushroom species. The transferfactor, which is a typical indicator of green plants, ranges between 0.03 and 0.05 this is by an order magnitude lower than in mushroom.

Total-beta activity

The average concentration of total-beta activity was found to be 1320 Bq kg^{-1} d.w. which comes primarily from the K-40 isotope. The Sr-90 activities of samples were very low, the average concentration was 2.1 Bq kg^{-1} d.w. lower three order of magnitude than the K-40 activities. Corresponding

Table 3
Total-beta and ^{90}Sr activities

Name	Total-beta act.			$^{90}\text{Sr} + ^{90}\text{Y}$ act.		
	Bq g ⁻¹ ash error σ		Bq kg ⁻¹ d.w.	mBq g ⁻¹ ash error σ		Bq kg ⁻¹ d.w.
Morchella esculenta	10.22	5.72	907	39.8	5.83	3.5
Helvella lacunosa	10.31	3.41	1009	10.5	6.41	1.0
Paxina leucomelas	10.55	4.73	1211	9.3	2.94	1.0
Laetiporus sulphureus	20.03	6.25	1627	8.1	0.42	0.5
Suillus granulatus '89	16.69	8.34	873	24.9	5.91	1.3
Suillus granulatus '90	11.53	5.12	1111	52.1	7.34	5.0
Omphalotus olearius	15.58	5.87	773	12.8	3.24	0.6
Tricholoma terreum '89	15.12	4.26	1102	17.6	4.62	1.3
Tricholoma terreum '90	10.88	3.51	2415	2.9	0.31	0.7
Tricholoma portentosum	15.02	4.22	1585	15.2	1.22	1.6
Lepista nuda	19.59	5.19	2175	24.7	4.83	2.7
Lepista sordida	10.72	3.78	1188	10.7	3.01	1.2
Lepista inversa	13.70	4.93	1175	34.4	7.92	4.5
Lepista gilva	12.30	4.32	1341	27.0	8.34	2.9
Armillariella mellea	8.06	2.01	1836	0		0
Collybia dryophila '89	12.50	4.51	1021	24.9	7.21	2.0
Collybia dryophila '90	12.40	8.22	929	29.2	5.62	2.2
Collybia butyracea	11.60	3.33	1543	18.8	3.54	2.5
Marasmius oreades	9.96	3.54	847	27.4	7.73	2.3
Entoloma clypeatum	13.98	4.72	1398	8.4	2.15	0.8
Amanita phalloides '89	12.84	5.67	1233	7.4	2.56	0.7
Amanita phalloides '90	12.77	2.54	1791	20.0	3.45	2.8
Agaricus arvensis	11.31	4.32	1576	15.3	7.25	2.1
Macrolepiota procera	28.63	6.01	1632	46.7	9.34	2.7
Macrolepiota rhacodes	6.77	2.71	1130	47.0	8.63	7.8
Lactarius vellereus	13.13	5.12	903	0		0

to the fact that the calcium and natural content of strontium in mushrooms is rather low.

Presence of Ag-110m

Among the 26 samples tested four were found to contain Ag-110m activities. Regarding the short half period of Ag-110m the silver founding mushrooms cannot be of Chernobyl origin. Rather it is likely that it is emitted from PWR type power plants under normal conditions. We did not find

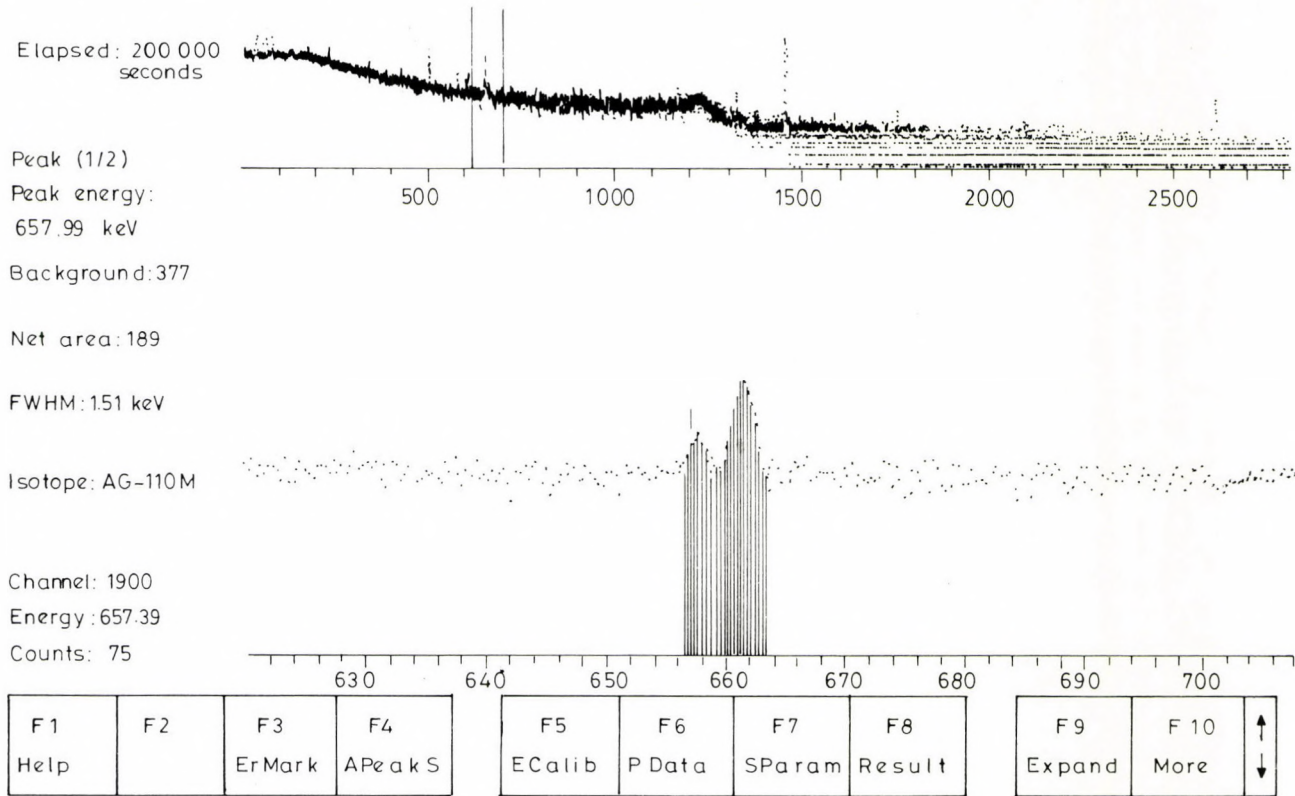


Fig. 1. A representative gammaspectrum of Macrolepiota procera sample

the Ag-110 m isotope in soil samples belonging to the mushrooms, suggesting that these mushroom species are able to selectively bioaccumulate the silver. Since the mushroom/soil transferfactor can reach a value of 500-1000 it is obvious that this value depends on the species of mushroom (ALLEN and STEINNE 1978). A representative gamma-spectrum of Macrolepiota procera sample with magnification of Ag-110m peak could be regarded in Fig. 1. Based on our measurements we point out that Macrolepiota species can be used as suitable tools to indicate the presence of the most likely appearing Ag-110m. Experiment are under way to work out such an indicator system.

REFERENCES

- Allen, R. O., Steinne, E. (1978): Concentrations of some Potentially Toxic Metals and Other Trace Elements in Wild Mushrooms from Norway. Chemosphere 4: 371-378.
- Benes, J., Kyrs, M. (1986): The Coprecipitation of Trace Amounts of Strontium with Barium Chromate. Collection Czechoslov. Commun. 33: 2822-2832.
- Dietl, G. (1989): Zur Verteilung Radioaktiver Caesiumnuklide im Pilzfruchtkörper. Z. Mykol. 55 (1): 131-134.
- Gans, J. (1987): Radionuklidkonzentrationen in Berliner Pilzen. Z. Mykol. 53 (1): 151-154.
- Grueter, H. (1971): Radioactive Fission Product Cs-137 in Mushrooms in Germany During 1963-1970. Health Physics. 20: 655-656.
- Haselwandter, K. (1978): Accumulation of the Radioactive Nuclide Cs-137 in Fruitbodies of Basidiomycetes. Health Physics. 34: 713-715.
- Horyna, J., Randa, Z. (1988): Uptake of Radiocesium and Alkali Metals by Mushrooms. J. Radioanal. Nucl. Chem., Letters 127 (2): 107-120.
- Molzahn, D., Reinen, D., Behr, H., Kocksholt, P., Patzelt, P. (1989): Die Belastung von Pilzen mit Radioaktivem Caesium. Z. Mykol. 55 (1): 135-148.
- Montag, A. (1965): Bestimmung von Strontium-89 und Strontium-90 in Lebensmitteln durch Erdalkalinsulfat-Trennung. Lebensm. Unters. Forsch. 127 (3): 121-130.
- Radiological Monitoring Service: Control of Radioactive Contamination of Food in Hungary in 1989.
- Radiological Monitoring Service: Control of Radioactive Contamination of Food in Hungary in 1990.
- Randa, Z. (1988): Radiocesium Tracer Obtained from Mushrooms. J. Radioanal. Nucl. Chem., Letters 126: 345-349.
- Rückert, G., Diehl, J. F. (1987): Anreicherung von Caesium-137 und Caesium-134 in 34 Pilzarten nach dem Reaktorunglück von Tschernobyl. Lebensm. Forsch. 185: 91-97.
- Steffan, B., Steglich, W. (1984): Die Hutfarbstoffe des Maronenröhrlings (Xerocomus badius). Angew. Chem. 96: 435-445.
- Teherani, D. K. (1987): Accumulation of Ru-103, Cs-137 and Cs-134 in Fruitbodies of Various Mushrooms from Austria after the Chernobyl Incident. Radioanal. Nucl. Chem., Letters 117 (2): 69-74.
- Vetter, J. (1987): Magasabbrendű gombák ásványianyag-tartalmának vizsgálata. Mikol. Közlem. 2-3: 125-150.

EFFECTS OF PHOSPHATE APPLICATION ON CARBOHYDRATE PARTITION AND CHLOROPLAST ULTRASTRUCTURE OF TWO CAPSICUM ANNUUM L. VARIETIES

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The plant dry mass, the sucrose, monosaccharide and starch content of the leaf and root, the malate content of the leaf were measured, and the chloroplast ultrastructure was studied on two varieties of *Capsicum annuum* L. plants grown at low (0.1 mmol dm^{-3}), medium (1.0 mmol dm^{-3}) and high (10 mmol dm^{-3}) H_2PO_4^- supplies. The low phosphate supply caused highly reduced biomass. The high supply increased the number of reproductive organs as compared to medium supply. Carbohydrate allocation was observed from leaf to root, so the sucrose and starch content of the root was relatively high, and that of the leaf greatly decreased at low phosphate supply. The plants at medium phosphate supply have the highest starch content in the leaves and monosaccharide content in the root. The effect of low phosphate supply was detectable on the chloroplast ultrastructure: the starch area ratio increased and the ratio of appressed to non-appressed membranes was the lowest here. The amount of appressed membranes was strikingly high at high phosphate application. The leaves contained $28-40 \mu\text{mol g}^{-1}$ malate. The malate content did not change in different phosphate supplies, but it showed a slight genetic variability.

Keywords: phosphate supply, biomass productivity, sucrose, starch, monosaccharide, malate, chloroplast ultrastructure

Introduction

Mineral nutrition of plants is one of the most important factors controlling biomass production (SARIC 1983). The insufficient availability of phosphorus causes decreased biomass production, plant height and leaf area (BARRETT-LENARD *et al.* 1982; MURALI and TERAMURA 1985; REPKA 1983), increased allocation to root (GERLOFF and GABELMAN 1983). The P_i uptake influences the uptake of other nutrients (SPIERS 1984), so a plant grown at suboptimal P_i supply shows general nutrient deficiency symptoms.

Abbreviations: CE and FE: 'Cecei' and 'Fehérözön' *Capsicum annuum* varieties; L, M, H: low, medial and high phosphate supply; P_i : inorganic phosphate.

There are good evidences that nutrient shortage causes injuries in the photosynthetic apparatus and consequently reduces the photosynthetic capacity (SAWADA et al. 1983; HUNT et al. 1985). Other data suggest that the rate of photosynthesis does not limit structural growing at suboptimal nutrient availability (MCDONALD et al. 1986; HAJIBAGHERI and FLOWERS 1985). This is possible in the cases where the low nutrient status caused increased starch content of the leaves (SAWADA et al. 1983; SELGA et al. 1983; MCDONALD et al. 1986; HAJIBAGHERI and FLOWERS 1985). The nutrient status influences the carbohydrate partition in connection with growing. The effect of P_i on carbohydrate partition is well known on cell level: a fine regulatory network exists in photosynthesizing cells, which allows adjustment of the rate of sucrose synthesis and the concentration of metabolites in the cytosol. P_i has a crucial role in this network (STITT and HELDT 1985). The concentration of P_i in cytosol drives the triosephosphate export of chloroplast via phosphate translocator; P_i has a role in ADP-glucose-pyrophosphorylase activation mechanism (PREISS 1982). Consequently P_i is an important regulator of starch metabolism. There are few data on the effects of nutritional status on carbohydrate partition on whole plant level (LAMBERS et al. 1981; HOFSTRA et al. 1985).

The aim of this paper is to compare the effects of sub- and supra-optimal phosphate application on carbohydrate partition in the leaves and root of Capsicum annuum plants. We detected the changes in the ultrastructure of the chloroplast in order to obtain informations about the status of photosynthetic apparatus.

The malate content of the leaf was measured, because earlier experiments showed that in the leaves of C3 Capsicum annuum species there is much more malate than in the leaves of C4 Zea mays species (TÉCSI et al. 1987). GERHARDT and HELDT (1984) demonstrated considerable malate content of C3 Spinacia oleracea leaves and diurnally fluctuating concentration. The function of this metabolite in C3 plants and its quantitative change under different environmental conditions is unknown. We have probed the effect of different phosphate application on the malate content of C. annuum plants in present experiments.

Material and Methods

Plant cultivation

Two varieties of *Capsicum annuum* L. plants, 'Cecei' and 'Fehérözön' (henceforth abbreviated as CE and FE, respectively) were grown in plastic pots (volume 600 cm³). The pots were filled with sand: perlite mixture (ratio 1:1), and contained 3 plants. The moisture content was kept at 70% of total water capacity of the mixture by daily watering with distilled water. The seeds were sown directly in the pots and at 2-week age 3 uniform plants were chosen per pot, others were removed. The plants were watered with 20 cm³ nutrient solution per pot twice a week. We applied three phosphate fertilization treatments: 0.1 (low), 1.0 (medium) and 10 (high) mmol dm⁻³ NaH₂PO₄ in a base nutrient solution. The concentrations of other nutrients were: 4 mmol K⁺, 5 mmol Ca²⁺, 1.2 mmol Mg²⁺, 1 mmol Cl⁻, 12 mmol NO₃⁻, 1.2 mmol SO₄²⁻, 1.6 mmol BO₃³⁻, 0.05 mmol MoO₄²⁻, 0.8 mmol Mn²⁺, 0.06 mmol Zn²⁺, 0.03 mmol Cu²⁺, 10 mmol Fe³⁺ per dm³ nutrient solution.

The plants were grown in a climate chamber, where the temperature was 21±2 °C day and night constantly, the photon flux density was 180 μmol m⁻²s⁻¹ providing by F33 TUNGSRAM fluorescent tubes, during a daily 16 h photoperiod.

Electronmicroscopic studies

For electronmicroscopic studies samples were taken from the central part of a recently expanded leaf of 70-day old plants. The leaf segments were fixed in Karnovsky fixative then contrasted by OsO₄, after dehydration in an alcohol series embedded in Durcupan ACM resin. The thin sections, cut by Reichert ultramicrotome, were stained with Pb-citrate.

The chloroplast ultrastructure was analysed from electron micrographs.

Measurement of biomass production

On the 70th day after sowing 12 plants per treatment and variety were harvested at the beginning of photoperiod. The fresh mass of leaves, roots and stems were measured separately, in 4 repetitions. The leaf area were measured with Tamaya Planix 8 planimeter. The parts of the plants were rapidly frozen in liquid the photoperiod. The published data are the average value of the two harvests. The lyophilized plant matter was weighed, then grinded and used for carbohydrate and malate analysis.

Carbohydrate analysis

The dry tissue was extracted with hot 70% (v/v) ethanol three times. The combined extracts were used for measuring the total soluble sugar content by colorimetric method (DUBOIS *et al.* 1956), using phenol and sulphuric acid. 10 cm³ of the ethanolic extract was alkalinized with 0.5 cm³ of 300 g dm⁻³ KOH, and kept on 90 °C for 30 minutes in order to destroy the monosaccharides (HANDEL 1968), then the remained sucrose was measured with the DUBOIS *et al.* (1956) method. The residual pellet of ethanolic extraction was extracted with 35 g dm⁻³ perchloric acid twice (McCREADY *et al.* 1950) and the carbohydrate content of combined extracts was measured with the DUBOIS *et al.* (1956) method, and was considered to be starch content. The sum of soluble sugar and starch content is referred to as total nonstructural carbohydrate (TNC) content.

Malate analysis

The lyophilized leaf material was extracted by 0.3 mol dm⁻³ perchloric acid (USUDA 1985). After removing the perchloric acid with potassium carbonate the malate content was measured enzymatically (HOHORST 1970).

Results

1. Biomass production

The most striking effect of low phosphate application is the biomass reduction. The plants grown at low phosphate supply accumulated five times

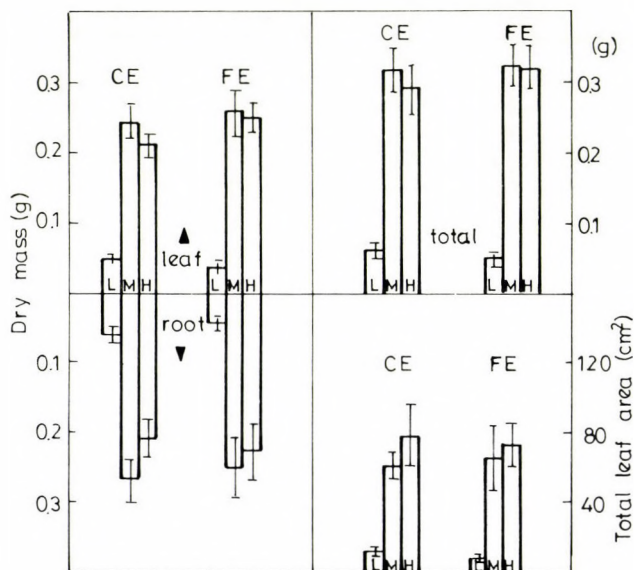


Fig. 1. The dry matter accumulation and total leaf area of two *Capsicum annuum* varieties (CE and FE) grown in low (L), medium (M) and high (H) phosphate supplies

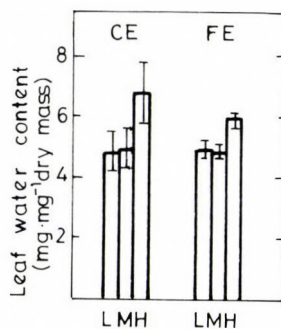


Fig. 2. Water content of the leaves on dry mass basis. *C. annuum* varieties: CE and FE. Phosphate supplies: low (L), medium (M) and high (H)

less leaf and root dry matter, and had six times less total leaf area (Figs 1, 2), than the plants at medium supply.

These plants had little, hard, fragile and dark green leaves. The high phosphate application caused only a slight decrease of dry matter, but increased the total leaf area and water content. These changes are significant (at $P=5\%$) only in the case of CE genotype.

The plants grown at low phosphate supply had no flowers at the harvest time, while the plants of higher supplies were at the beginning of reproductive period. There were much more flowers on the plants at high phosphate supply than at medium supply, but the quantitative evaluation was impossible.

2. Carbohydrate partition

The sucrose content of the leaves increased with increasing phosphate supply. The decrease caused by low phosphate application is nearly equal to the increase caused by high application: 10-15%. The change of root sucrose concentration is opposite to the change of leaf sucrose concentration (Fig. 3). The soluble sugars fraction destroyable by heating in alkaline medium is referred to as monosaccharides.

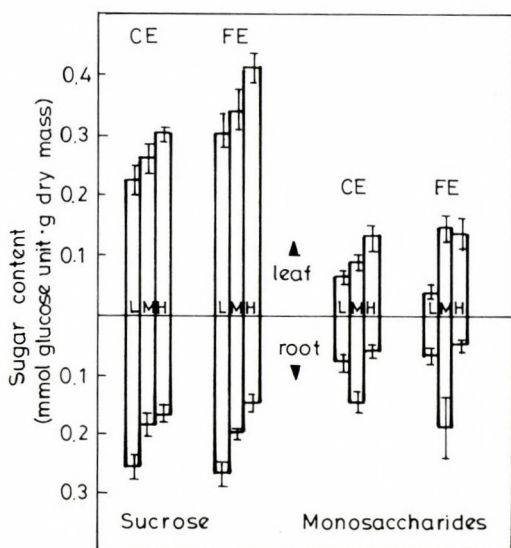


Fig. 3. Sugar content of the leaves and root of two *C. annuum* varieties (CE and FE) grown in low (L), medium (M) and high (H) phosphate supplies

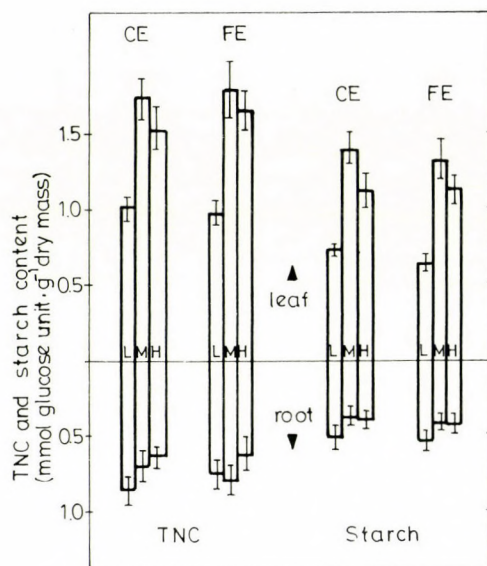


Fig. 4. TNC (= soluble sugars+starch) content and starch content of the leaves and root of two *C. annuum* varieties (CE and FE) grown in low (L), medium (M) and high (H) phosphate supplies

The change of this fraction in the leaf, caused by different phosphate supplies was parallel with starch content in CE variety, and parallel with starch content in FE variety. There is a strikingly high monosaccharide content of the root at medium phosphate supply as compared with the low and high supplies (Fig. 3).

The starch content of the leaves decreased both at the low and high phosphate supply, but the decrease caused by phosphate deficiency is much sharper than that of high phosphate deficient plants, is nearly equal to the leaf starch content, but it is merely 30-40% of the leaf starch content at medium and high supplies (Fig. 4).

The changes of total nonstructural carbohydrate content of the leaves are highly parallel with the changes of starch content. The different phosphate supplies caused only little changes in the root TNC content (Fig. 4).

3. Chloroplast ultrastructure

The chloroplast structure of plants grown at medium and high phosphate application is similar in some aspects. The number of grana was 30-35 per $1 \mu\text{m}^2$ of the chloroplast section area. The degree of thylakoid aggregation

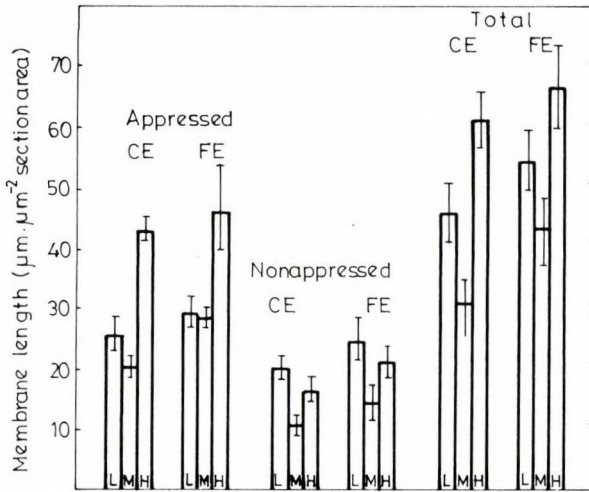


Fig. 5. Data of thylakoid membranes of the chloroplasts of two *C. annuum* varieties (CE and FE) grown in low (L), medium (M) and high (H) phosphate supplies. The sections for electron micrographs were made from a recently developed leaf of plants

was similar as well: 10-15 thylakoids per granum, there was no large columnal granum. The loculi were narrow, the thylakoids were not swollen. The chloroplasts of the plants at high phosphate supply contained much more appressed membranes than the plants at medium supply. The total length of membranes (Fig. 5) and the ratio of appressed to non appressed membranes (Table 1) were the highest at high supply.

The shape of chloroplast sections at low phosphate supply was nearly round. The main differences were the followings, as compared to chloroplasts

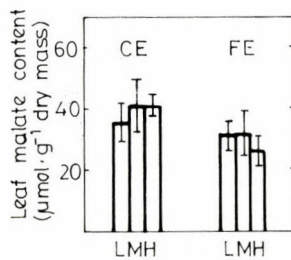


Fig. 6. Malate content of the leaves of two *C. annuum* varieties (CE and FE) grown in low (L), medium (M) and high (H) phosphate supplies

Table 1

Ratio of appressed to nonappressed membranes of the chloroplasts of two *Capsicum annuum* varieties (CE and FE) grown in low (L), medium (M) and high (H) phosphate supplies. The measurements was made on electron micrographs of a recently expanded leaf of the plants

Phosphate supply	Ratio of appressed to nonappressed thylakoid membranes		Area of starch grains	
	(μm μm ⁻¹)		(percentage of chloroplast area)	
	CE	FE	CE	FE
L	1.31	1.23	43.9	45.2
M	2.00	2.10	27.9	40.0
H	2.59	2.24	33.3	34.9

at higher phosphate supplies: The starch area ratio was higher (43-45%, Table 1), the number of grana was fewer (20-25 per 1 μm² of chloroplast section area), but there were more larger grana (consisting of 20-25 thylakoids) among them. These chloroplasts contained more stroma plasma and stroma membrane. The ratio of appressed to nonappressed membranes was the lowest here. The low phosphate supply caused a moderate thylakoid deformation: some membranes were waved and loculi swollen.

4. Malate content

The malate content was 38-40 and 28-30 μmol g⁻¹ dry matter of leaves of CE and FE genotypes, respectively. The different phosphate application caused no significant changes in malate content.

Discussion

The detected effects caused by different phosphate application are fairly similar at the two *C. annuum* varieties. So the two varieties can be considered as controls of each other, and it is suggested that the findings may be general within the species. Watering with a solution containing merely 0.2 mmol dm⁻³ H₂PO₄⁻ caused a very severe nutritional stress. This is suggested by the hard decrease in biomass and leaf TNC content. The chloroplast ultrastructure shows the typical signs of phosphate deficiency: more non-appressed membranes and starch grains, swollen loculi few granum, as described THOMSON *et al.* (1964) and WATHLEY (1971).

The plants at medium phosphate supply show some symptoma of mild phosphate deficiency: higher leaf starch content (being measured by chemical method) few thylakoid membranes in chloroplasts. It is well documented that the low nutrient availability causes increased starch content of the leaves (SAWADA et al. 1983; SELGA et al. 1983; MCDONALD et al. 1986). Our results indicate that extremely low and long-lasting phosphate supply does not cause an increased starch content of the C. annuum leaves, but do cause increased sucrose and starch content of the root.

The data of dry mass (Fig. 1) show that the plants at high phosphate supply did not develop more vegetative organs, but the detected differences in water content of the leaf, chloroplast ultrastructure and carbohydrate content indicate the qualitative changes caused by excess phosphate. Perhaps the excess phosphate affords chance for more vigorous reproductive development, for the plants have more flower at high phosphate supply.

Although there are detectable changes in chloroplast ultrastructure of plants grown at low phosphate supply, unlikely, that it was the rate of photosynthesis which limited the structural growing, as MCDONALD et al. (1986) concluded too, studying nitrate deficient small birch plants. In our experiment the sucrose and starch content of the root was relatively high at low phosphate supply, perhaps it was enough for growing, but the root, and consequently the shoot could not grow because of the lack of other metabolites containing phosphorus. The sucrose and starch contents of the leaves and root clearly shows an allocation from leaf to root at low phosphate supply. It is a sign of the compensation mechanism which may reduce the negative effects of low nutrient availability (GERLOFF and GABELMAN 1983).

There is a contradiction between the higher starch area ratio of chloroplasts, observed by electronmicroscope, and the lower starch content of the leaf, measured by chemical method, in phosphate deficient plants. The possible reason of the contradiction is that the samples derives from a middle positioned leaf for ultrastructural studies, but we used the combined dry matter of all leaf position for chemical analysis. But may be, that the severe deficient plants have few starch reserve in the leaf, because of reduced photosynthetic capacity, and most of starch, localised in the chloroplasts, is unable to get out of it. Getting out of triose-phosphates, formed of chloroplastic starch, is phosphate dependent (PREISS 1982). The highly increased root monosaccharide content at medium phosphate supply is a

remarkable result. Further investigations are needed to discover its importance and reason.

The data of leaf malate content verified the earlier findings that the leaf of *C. annuum* contains about five times more malate than maize leaves (TÉCSI et al. 1987). Though the malate content of the leaves did not change significantly with the different phosphate supplies, it shows a slight genetic variability.

REFERENCES

- Barrett-Lenard, E. G., Robson, A. D., Greenway, H. (1982): Effect of phosphorus nutrition deficiency and water deficit on phosphatase activities from wheat leaves. *J. Exp. Bot.* **33**: 682–693.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Roberts, D. A., Smith, E. (1956): Colorimetric determination of sugar and related substances. *Anal. Chem.* **28**: 350–356.
- Gerhardt, R., Heldt, H. (1984): Measurement of subcellular metabolite levels by fractionation of freezestopped material in nonaqueous media. *Plant Physiol.* **75**: 542–547.
- Gerloff, G. C., Gabelman, W. H. (1983): Genetic basis of inorganic plant nutrition. In: Lauchli, A., Bielski, R. L. (eds): *Encyclopedia of Plant Physiology*. Vol. 15. B. Springer-Verl., Berlin.
- Hajibagheri, M. A., Flowers, T. J. (1985): Salt tolerance in the halophyte *Suaeda maritima* (L.) Dum. The influence of salinity of the culture solution on leaf starch and phosphate content. *Plant Cell and Environment* **8**: 261–267.
- Handel, E. (1968): Direct microdetermination of metabolism of *Urtica dioica* as dependent on the supply of mineral nutrients. *Physiol. Plant.* **63**: 13–18.
- Hohorst, H. J. (1970): L(–)-Malat Bestimmung mit Malatdehydrogenase und NAD. In: Bergmeyer, H. U. (ed.): *Methoden der enzymatischen Analyse*. Acad. Verl. Berlin, 1544–1548.
- Hunt, E. R., Weber, J. A., Gates, D. M. (1985): Effects of nitrate application on *Amaranthus powellii* Wats. *Plant Physiol.* **79**: 609–624.
- Labbers, H. (posthumus), Stulen, I., Lanting, S. J., van de Dijk, R., Hofstra, R. (1981): Energy metabolism of *Plantago lanceolata* as dependent on the supply of mineral nutrients. *Physiol. Plant.* **51**: 85–92.
- McCready, R. M., Guggolz, J., Silveira, V., Owens, H. S. (1950): Determination of starch and amylase in vegetables. *Anal. Chem.* **22**: 1156–1158.
- McDonald, A. J. S., Ericson, A., Lomammar, T. (1986): Dependence of starch storage on nutrient availability and photon flux density in small birch. *Plant Cell and Environment* **9**: 433–438.
- Murali, N. S., Teramura, A. H. (1985): Effects of ultraviolet-B irradiance on soybean. VII. Biomass and concentration and uptake of nutrients at varying P supply. *J. Plant Nutr.* **8**: 177–192.
- Preiss, J. (1982): Regulation of the biosynthesis and degradation of starch. *Ann. Rev. Plant Physiol.* **33**: 431–454.

- Repka, J. (1983): Accumulation of ions in the leaves and chloroplasts, photosynthesis and growth of maize plants under deficiency and excess of nitrogen, phosphorus and potassium in the medium. Photosynthetica 17: 371—378.
- Saric, M. R. (1983): Theoretical and practical approaches to the genetic specificity of mineral nutrition of plants. In: Saric, M. R., Loughman, B. C. (eds): Genetic Aspects of Plant Nutrition, Martinus Nijhoff The Hague, pp. 1—17.
- Savac̆, S., Igarashi, T., Miyachy, S. (1983): Effects of phosphate nutrition on photosynthesis starch and total phosphorus levels in single rooted leaf of dwarf bean. Photosynthetica 17: 484—490.
- Selga, M., Vitiola, A., Kristkalne, S., Gubar, G. B. (1983): Accumulation of photosynthates in chloroplasts under various irradiances and mineral nutrition of Cucumber plants. Photosynthetica 17: 171—175.
- Spiers, J. M. (1984): Elemental leaf content and deficiency symptoms in rabbiteye blueberries. 3. Phosphorus and potassium. J. Plant Nutr. 7: 1567—1581.
- Stitt, M., Heldt, H. W. (1985): Control of photosynthetic sucrose synthesis by fructose-2,6-biphosphate. Plant Physiol. 79: 599—608.
- Técsi, L., Margóczy, K., Maróti, I. (1987): Distribution of photosynthates in the leaves of two maize lines in the initial phase of the photoperiod. Acta Bot. Hung. 33: 421—430.
- Thomson, W. W., Weier, T. E., Drever, H. (1964): Electron microscopic studies on chloroplasts from phosphorus deficient plants. Am. J. Bot. 51: 933—938.
- Usuda, H. (1985): Changes in levels of intermediates of the C4 cycle and reductive pentose-phosphate pathway during induction of photosynthesis in maize leaves. Plant Physiol. 78: 859—864.
- Wathley, J. M. (1971): Ultrastructural changes in chloroplasts of Phaseolus vulgaris development under conditions of nutrient deficiency. New Phytol. 70: 725—742.

FUNGAL SPOILAGE OF FRESH PEPPER CAPRICUM ANNUUM L.

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Fresh pepper fruits were experimentally infected with spores of Aspergillus flavus, Botryodiplodia theobromae and Rhizopus oryzae to determine the mode of entry of the organisms into pepper tissues. The fungi gained entrance through wounds and point of attachment of the stalk to the fruit. The fungi caused spoilage (rots) of the fruits at between 10 °C and 40 °C, at higher temperature, fruits started to dehydrate and infection could not be determined.

The amounts of mycelia produced by each of the fungi when inoculated into the fruit extract was directly proportional to the concentration of the extract. Our findings suggest that adequate protection from injuries during harvesting, and storage at low temperatures are very useful adjuncts in the prevention of microbial spoilage of pepper fruits.

Introduction

Post-harvest spoilage of pepper (Capsicum) has been reported in Nigeria (ADISA 1980). A number of microorganisms have been implicated as major spoilage agents of the fruits. BARTZ and STALL (1974) reported that different species of pepper fruits infected with Erwinia caratovora showed signs of rot within 48 h. Corynebacterium michiganense was also reported to infect pepper (DEHLBECK et al. 1979).

Fungal soft rot of pepper caused by Pythium debaryanum was documented as far back as 1922 by LEHMAN. In Nigeria, ADISA (1980) identified Aspergillus aculeatus, Rhizopus oryzae, A. fumigatus, Fusarium equiseti and F. oxysporum as responsible for dry rots of pepper.

In this paper the results of the mode of entry, effect of temperature on rot development, the effect of different concentrations of pepper extract

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on mycelia production of the isolates as well as the effect of different treatments on disease development are reported.

Material and Methods

Determination of the mode of entry of the fungi

Healthy fresh fruits were rinsed in sterile distilled water and surface sterilized with 70% ethanol. The fruits were grouped into two. In the first group, a laceration was made on the surface of each fruit by means of a sterile scalpel while the fruits in the second group were left unwounded. Fruits in both groups were dipped into a spore suspension (20×10^6 spores/ml) of the appropriate fungus. Each fruit was transferred aseptically into a transparent polyethylene bag containing sterile wet cotton plugs to create a high micro-humidity chamber. The bags were sealed and incubated at 30 °C. For controls, unwounded and wounded fruits were surface sterilized and dipped into autoclaved spore suspension of the appropriate fungus. Each control fruit was also transferred into a transparent polyethylene bag, marked and incubated along with the others. The fruits were observed daily for rot symptoms.

Effect of temperature on rot development

Healthy fruits were washed and surface sterilized with 70% ethanol. One laceration was made on the surface of each fruit with a sterile scalpel. Into the wound was inoculated 1 ml of the spore suspension of the appropriate fungus. Each fruit was transferred aseptically into a transparent polyethylene bag containing wet cotton plugs. The bags were sealed and ten bags were incubated at each of the following temperatures, 5, 10, 20, 30, 35, 40 and 50 °C for 7 days. The fruits were checked daily for rot symptoms.

Effect of different concentrations of pepper extract on mycelial growth of organisms

Four hundred grammes of pepper were blended in 100 ml of distilled water and filtered by means of Buckner's funnel using a vacuum pump. Portions of the filtrate were serially diluted to give suspensions of the following concentrations 1, 5, 10, 20 and 40%. These were dispensed in 30 ml aliquots into 150 ml-flasks and sterilized at 121 °C for 15 minutes.

On cooling, each flask was inoculated with a 5 mm diameter of agar and mycelia disc of the appropriate fungus and incubated at 30 °C for 7 days. Growth was assessed by the dry weight method.

Effect of different treatment on disease development

Pepper fruits were washed and packed in groups of ten and treated as follows:

- (i) Rinsing in distilled water and stored at 30 °C and 10 °C, respectively
- (ii) Rinsing in 10% milton (sterilizing solution) and stored at 10 °C
- (iii) Rinsing in 10% milton (sterilizing solution) and stored at 5 °C in an RH chamber of 59%
- (iv) Rinsing in 10% milton solution and stored at 5 °C in an RH chamber of 75%

Results

Results of experiments showed that the mode of entry of the three spoilage fungi into the tissues of pepper fruits was through wounds (or injuries) and the point of attachment of the stalk to the fruit. The pene-

Table 1

Mode of entry of isolates into pepper tissue

Organism	Route		
	Point of attachment	Laceration	Unbroken cuticle
<i>Aspergillus flavus</i>	-	+	-
<i>Botryodiplodia theobromae</i>	+	+	-
<i>Rhizopus oryzae</i>	+	+	-

+: indicates that organisms were able to penetrate and cause rots

-: no penetration observed

tration occurred whether or not the stalk was still firmly attached to the fruits for *B. theobromae* and *R. oryzae* (Table 1).

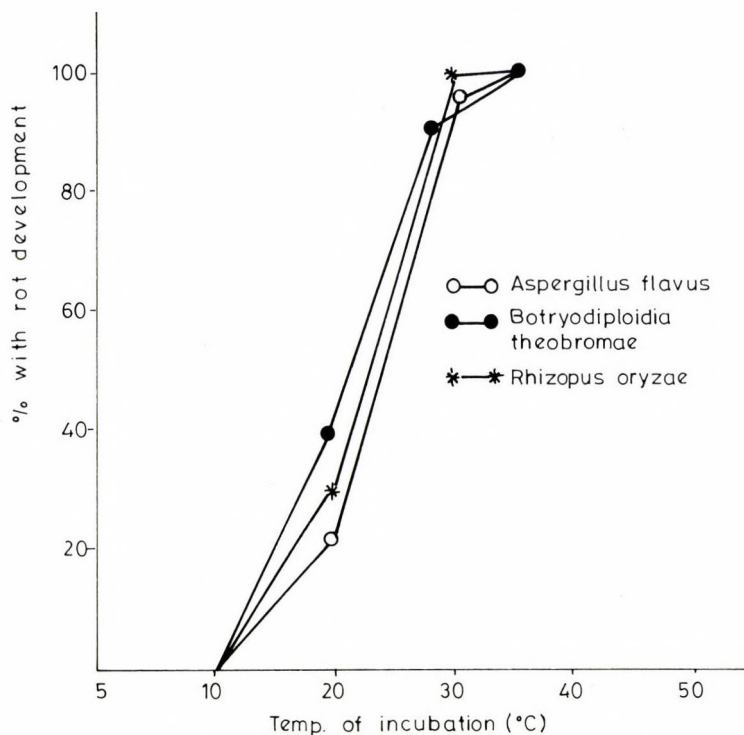


Fig. 1. Effect of temperature on rot development of fruits infected with spoilage fungi

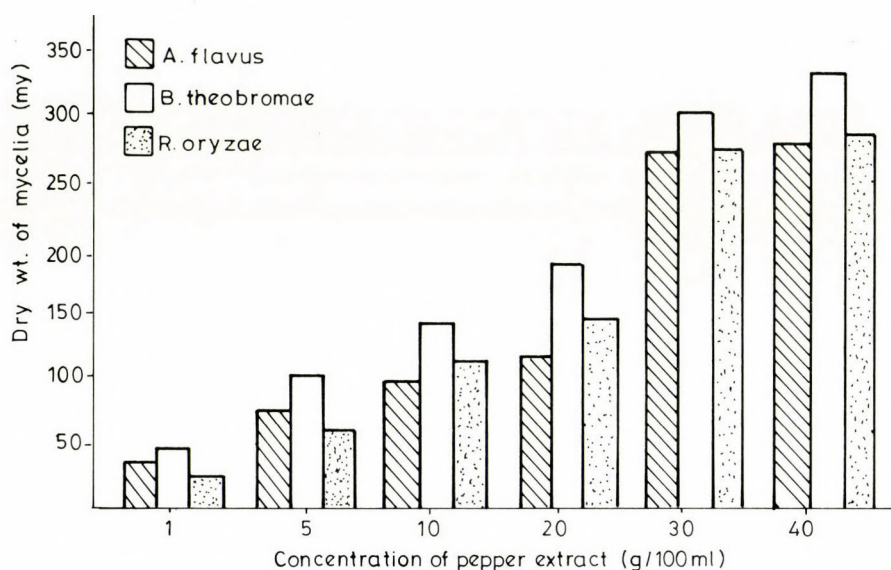


Fig. 2. Effect of different concentrations of pepper extract on the growth of the spoilage fungi

No rots occurred at 5 °C and 10 °C on fruits inoculated with each of the three organisms during the period of incubation. All fruits showed signs of rot at 30 °C and 35 °C for all isolates (Fig. 1). The higher the concentration of pepper extract the higher the mycelia mass produced by each

Table 2

Effect of different treatments on disease development.
Figures are a mean of three readings \pm SD

Treatment	Temperature of incubation (°C)	Onset of rot development (days)
(1) Rinsing with water only	30	6 \pm 1
(2) Rinsing with water only	10	25 \pm 3
(3) Rinsing in 10% milton solution	10	35 \pm 2.5
(4) Rinsing in 10% milton solution and storage at 59% RH	5	35 \pm 95
(5) Rinsing in 10% milton solution and storage at 75% RH	5	35 \pm 0.8

of the isolates. While 1% pepper extract solution supported less than 50 mg of fungal mycelia, 40% pepper extract solution supported 330 mg mycelia growth for B. theobromae (Fig. 2).

Results of experiments showed that fresh pepper fruits washed with distilled water and air-dried stored for only 5-7 days, fruits washed and stored at 10 °C (showed signs of rot development on the 25th day). Rinsing with 10% milton solution followed by storage at 5 °C or 10 °C at either 59% or 79% RH delayed onset of rot for 35 days (Table 2).

Discussion

The lack of infection observed when spores were smeared on unbroken pepper skin is probably due to the fact that the enzymes produced by the test fungi were not capable of dissolving the protective cuticle of the pepper fruit. Infection through the scar of attachment of the stalk (Table 1) suggests that the tissues in this region are unprotected and therefore susceptible to attack by the organisms. Post-harvest spoilage of pepper arise mainly from microbial infection of wounds sustained during handling of the crop. The fruits are harvested into large open baskets by the local farmers and often piled on top of one another or put into fibre-bags. In either case, the fruits receive very little protection from injuries which indeed occur extensively. The wounds readily become colonised by propagules of pathogens contained in fluids leaking from already rotted fruits. HARTER and WEIMER (1923) observed that Rhizopus species could not infect potato through its unbroken skin except through wounds and through weak points such as the stomata and lenticels (SMITH and RAMSEY 1947).

Rot development on fruits was highest at 30 °C and at 35 °C (Fig. 1) which corresponded to the optimum temperature for the mycelial growth of the isolates (EKUNDAYO 1985). Below these temperatures rot development on the fruits was reduced, and above these temperatures the fruits gradually dried up to loss of water.

The continued increase in the dry weight of the mycelia of all the test fungi with increase in the concentration of pepper extract (Fig. 2) supports the fact that the nutrients in pepper are in the available form for absorption and utilization by the microorganisms.

In the light of the results obtained in the study, the storage of fruits at low temperatures as practised by most people is highly recommended. The method of drying pepper fruits as practised by the local women

in Nigeria should also be encouraged as this leads to considerable reduction in the moisture content of the fruits there by reducing rate of infection by microorganisms.

In conclusion, we like to stress the need to avoid damages or injuries to the body of the fruits during harvesting and for farmers to ensure that the stalk or part of it is harvested along with the fruits in order to reduce the possibility of infection. Moreover, the storage of fresh fruits at low temperatures with a view to minimising microbial activities cannot be overemphasized.

REFERENCES

- Adisa, V. A. (1980): Post-harvest rots of some fruits with particular reference to Citrus species in S.W. Nigeria. Ph.D. thesis, Univ. of Ibadan, Nigeria.
- Bartz, F. A., Stall, W. M. (1974): Tolerance of fruits from different pepper lines to Erwinia caratovora. Phytopath. 64: 1290—1293.
- Dehlbeck, D., Stall, R. E., Jones, J. P. (1979): The effect of vertical and horizontal resistance on development of bacterial spot of pepper. Pl. Dis. Rep. 63: 332—355.
- Ekundayo, A. O. (1985): Post-harvest fungal spoilage of pepper (Capsicum annum L.). Ph.D. thesis, Univ. of Ibadan, Nigeria.
- Harter, L. L., Weimer, J. L. (1923): The relation of the enzymes pectinase to infection of sweet potatoes by Rhizopus. Am. J. Bot. 10: 245—258.
- Lehman, S. G. (1922): Soft rot of pepper fruits (Capsicum annum). Phytopath. 2: 85.
- Smith, M. A., Ramsey, G. B. (1947): Bacterial Lenticel infection of early potatoes. Phytopath. 37: 225—242.

EFFECT OF THE CALMODULIN-INHIBITOR, CHLORPROMAZINE ON BENZYLADENINE-INDUCED SHOOT FORMATION IN DEROOTED TOBACCO PLANTS

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Effect of a neuroleptic drug, chlorpromazine (CP) known to bind to calmodulin in a calcium-dependent manner and inhibits its function, was investigated on shoot and root formation of root-excised tobacco (*N. tabacum* L. cv. *Xanthi*) plants. CP inhibited in a higher degree the benzyladenine- (BA-) induced shoot formation, than the development of roots, regardless of the BA and CP concentrations, applied and of the actual ratio of shoot mass to root mass. In the absence of BA, CP treatment has no effect on root formation.

These results suggest that the induction of shoot and probably root formation by BA might be signalled by the Ca^{2+} -calmodulin system.

Introduction

One of the major mechanisms, by which primary outer signals arising from hormones, growth factors and neurotransmitters are transduced into animal cells is the calcium second messenger system (BERIDGE 1986). This pathway involves the activation of phospholipase C resulting in the hydrolysis of phosphatidylinositides to give rise to the formation of diacylglycerol and inositol triphosphate (IP_3). The latter causes release of Ca^{2+} from intracellular stores (first of all from endoplasmic reticulum). The pathway then involves the protein phosphorylating enzyme, protein kinase C, a kinase that is dependent both on calcium and (lyso-)phospholipids for activity, together with, at physiological concentrations of Ca^{2+} , diacylglycerol. In addition, the increased cytoplasmic Ca^{2+} through the activation of calcium/calmodulin-dependent kinase, is responsible for the phosphorylation of a different sub-set of proteins. The two sets of phosphorylated proteins (dual signal pathway) together are leading to the subsequent cellular response (KIKKAWA *et al.* 1986).

Many components of this dual pathway have been recently identified in plants (BOSS and MASSEL 1985; ELLIOTT and SKINNER 1986; SANDELIUS and SOM-

MARIN 1986; OLÁH and KISS 1986). The Ca^{2+} -dependent regulatory protein, calmodulin, was also purified from plant sources (MARMÉ 1983). However, there have been fewer reports on the interaction of the components of the pathway with different external stimuli (i.e. plant hormones, pathogens etc.) (MORSE *et al.* 1989).

For example KUROSAKI *et al.* (1987) reported that calmodulin inhibitors depress, while activators of protein kinase C could induce the elicitor-induced phytoalexin accumulation in carrot cells. Certain lines of evidence suggest that Ca^{2+} may be involved in the elicitation of bacterially induced hypersensitive response of plant cells, since the products of oxy free radical mediated lipid peroxidation (fatty acid hydroperoxides) and of phospholipid hydrolysis (phosphatidic acid) may serve as Ca^{2+} -ionophores (ÁDÁM *et al.* 1989; KEPPLER and BAKER 1989). Taken together, the convincing proof of their role in plant resistance is still lacking (STRASSER *et al.* 1986; KENDRA and HADWIGER 1987).

In this paper the role of Ca^{2+} -calmodulin system in the benzyladenine-induced developmental processes (shoot and root formation) of derooted tobacco is discussed.

Material and Methods

The root system of tobacco plants having 8-10 fully expanded leaves, were excised. The rootless tobacco stems were placed into 250 ml Erlenmeyer flasks. The flasks were covered with aluminium foil to prevent chlorpromazine (2-chloro-10-(3-dimethylaminopropyl)phenothiazine, CP) from light-induced degradation. The plants were incubated in ordinary greenhouse conditions for 10 days in the solutions described in Table 1.

Table 1

Benzyladenine (BA) and chlorpromazine (CP) contents of the incubation solutions¹

	BA (μM)			
	0	0.25	0.50	1.00
CP (μM)				
0	+	+	+	+
25	+	+	+	+
50	+	+	+	+

¹BA and CP were applied in Hoagland solution containing 284 mg kg^{-1} nitrate nitrogen (CHEO *et al.* 1952). The incubation solutions (200 ml) were replaced in every three days.

The newly formed shoots and roots were cut and the differences in the development of plants were characterized by their fresh mass per plant.

Chemicals used were reagent grade. Chlorpromazine was a gift from Chinoin Pharmaceutical Co. (Hungary). Benzyladenine (BA) was from Sigma, the other chemicals were purchased from Reanal Chemical Co. (Hungary).

Results and Discussion

Treatment with BA of rootless tobacco plants promoted the shoot formation (Figs 1 and 2). The shoot mass increased gradually with elevating concentration of BA. In nontreated (control) plants shoots were not formed, only roots developed (Fig. 1). The development of root system showed a less clear picture at different BA concentrations than shoot formation: 25 μM BA decreased but 50 μM enhanced root formation as compared to nontreated control ones. The ratio of shoot mass to root mass was the highest at 1 μM BA concentration (Table 2).

It has been well known for a long time that cytokinins in derooted systems can often substitute completely for roots in inducing a response in the shoot. The responses are different but concern first of all vegetative growth including lateral and adventitious bud outgrowth (shoot formation),

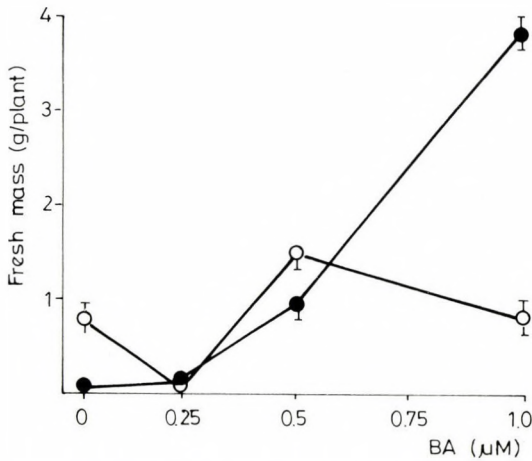


Fig. 1. Effect of benzyladenine (BA) on root and shoot formation of derooted tobacco plants. (○) and (●) are fresh mass (g/plant) of newly formed roots and shoots, respectively. The incubation medium contained 284 mg kg^{-1} nitrate nitrogen in Hoagland solution. (Vertical bars are means \pm SE, $n=6$ of two independent experiments. Where not indicated, bars were smaller than the marks)

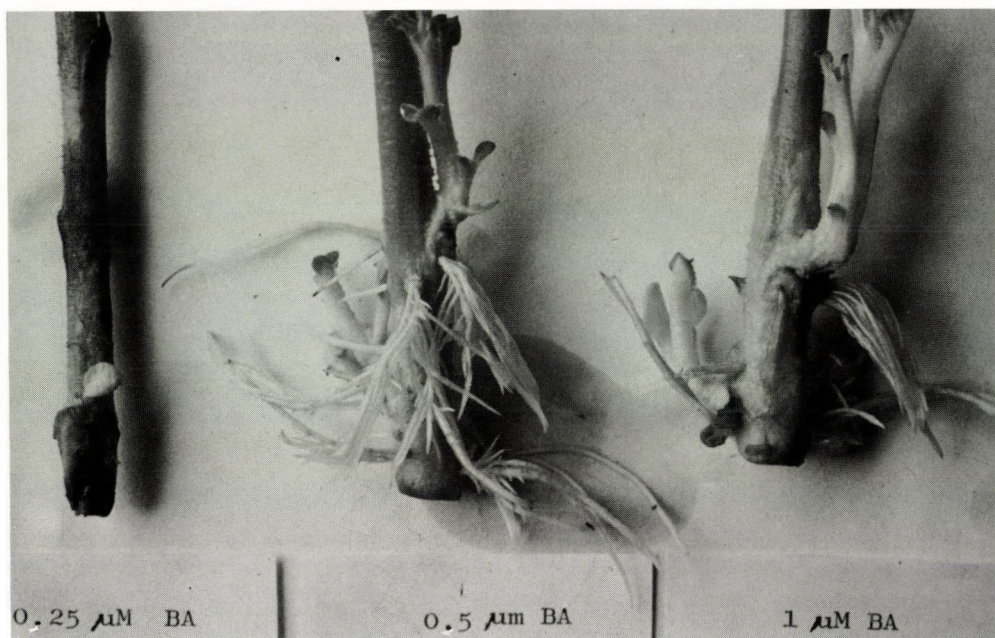


Fig. 2. Effect of benzyladenine (BA) on shoot and root formation of root-excised tobacco plants

inflorescence development and retardation of senescence. On the contrary, cytokinins generally inhibit lateral root production in intact roots (LESHEM *et al.* 1978). This latter effect was obvious only at low BA concentration (Fig. 1) where the shoot induction was also very weak.

Table 2

Effect of chlorpromazine (CP) on the ratio of shoots to roots in benzyladenine (BA) treated derooted tobacco plants¹

	BA (μ M)			
	0	0.25	0.50	1.00
CP (μ M)				
0	⁻²	3.37	0.64	4.75
25	⁻²	⁻²	0.55	3.71
50	⁻²	⁻²	0.40	2.07

¹Data presented, were calculated from data seen in Figs 1 and 3.

²Data could not be calculated because shoots were not formed.

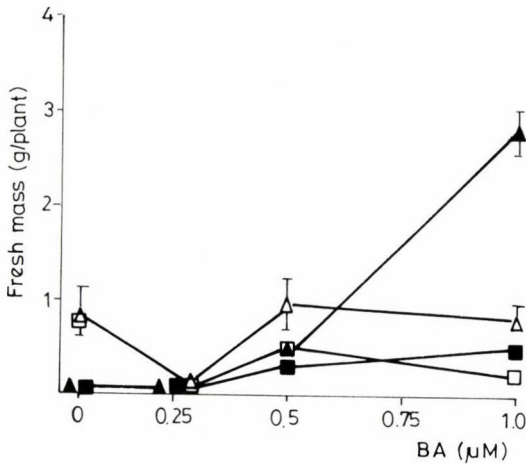


Fig. 3. Effect of chlorpromazine (CP) on benzyladenine- (BA-) induced root and shoot formation of root-excised tobacco plants. Open symbols are fresh mass (g/plant) of newly developed roots; closed symbols are fresh mass (g/plant) of newly formed shoots. (Δ , \blacktriangle) and (\square , \blacksquare) refer to plants treated with 25 and 50 μM CP, respectively. The incubation medium contained 284 mg kg^{-1} nitrate nitrogen in Hoagland solution. (Vertical bars are means \pm SE, $n=6$ of two independent experiments. Where not indicated, bars were smaller than the marks.)

CP treatments (25 and 50 μM) have no effect on root mass of BA-untreated (control) plants. However, in BA-treated plants CP depressed both root and shoot formation, on a concentration dependent manner (Figs 3 and 4). The inhibitory effect was more pronounced in plants treated with high concentrations of BA and CP. Root formation was less sensitive to CP treatments than shoot development in each combination. This is well demonstrated in Table 2, where the shoot/root ratios of plants of different treatments are compared. Due to CP treatment (50 μM) the ratio of shoot mass to root mass decreased to half of the one of control plants treated only with BA (1 μM) (Table 2).

It is well established that phenothiazines, among others the CP, bind to the hydrophobic domain of calmodulin and thus block the action of Ca^{2+} -calmodulin system (WEISS *et al.* 1980). Our results suggest that Ca^{2+} -calmodulin system could be involved in the developmental response to BA of de-rooted tobacco plants. However, the ineffectiveness of CP on derooting in the absence of BA (where shoots were not formed) and the decreased sensitivity of root formation to CP in the presence of BA (where shoot formation was induced) may indicate that calmodulin can influence root development only in the presence of BA or/and via shoot induction.



Fig. 4. Effect of chlorpromazine on benzyladenine- (BA-) induced shoot and root formation of root-excised tobacco plants

Role of dual signal pathway was also studied in other cytokinin-induced responses. It was found that the increase in both cytoplasmic Ca^{2+} -concentration and diacylglycerol level (ELLIOTT and PETKOFF 1989), as well as the Ca^{2+} -calmodulin system (ELLIOTT 1983) are involved in the BA-induced betacyanin synthesis in *Amaranthus* seedlings. Involvement of calmodulin and of changes in inositol phospholipid turnover in BA-induced cell division was also characterized (CONNETT and HANKE 1987; DAS *et al.* 1987). OLÁH *et al.* (1983) reported on the role of BA in the coupling between calmodulin and Ca-ATPase.

Auxin also stimulates IP_3 turnover (ETTLINGER and LEHLE 1987). Responses induced by abscisic acid and gibberellic acid were also found to be regulated by Ca^{2+} -calmodulin system or protein kinase C (ELLIOTT *et al.* 1983; LADYZHENSKAYA *et al.* 1987). Taken together, it is obvious, that the same signal pathway is involved in different hormone-induced responses. However, the basis for specific characteristics of these responses, i.e. activation of different genes is still less clear.

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REFERENCES

- Ádám, A., Farkas, T., Somlyai, G., Hevesi, M., Király, Z. (1989): Consequence of O_2^- generation during a bacterially induced hypersensitive reaction: deterioration of membrane lipids. Physiol. Mol. Plant Pathol. **34**: 13–36.
- Beridge, M. J. (1986): Inositol triphosphate and calcium mobilization. In: Evered, D., Whelan, J. (eds): Calcium and the Cell. Ciba Foundation Symp. 122. John Wiley and Sons, Chichester, pp.39–57.
- Boss, W. F., Massel, M. (1985): Polyphosphoinositides are present in plant tissue culture cells. Biochem. Biophys. Res. Commun. **132**: 1018–1032.
- Cheo, P. C., Pound, G. S., Weathers, L. G. (1952): The relation of Cucumber virus 1 in spinach. Phytopathology **42**: 377–381.
- Connett, R. J. A., Hanke, D. E. (1987): Changes in the pattern of phospholipid synthesis during the induction by cytokinin of cell division in soybean suspension cultures. Planta **170**: 161–167.
- Das, R., Bagga, S., Sopory, S. K. (1987): Involvement of phosphoinositides, calmodulin and glyoxalase-I in cell proliferation in callus cultures of Amaranthus paniculatus. Plant Sci. **53**: 45–51.
- Elliott, D. C. (1983): Inhibition of cytokinin-regulated responses by calmodulin-binding compounds. Plant Physiol. **72**: 215–218.
- Elliott, D. C., Batchelor, S. M., Cassar, R. C., Marinods, N. C. (1983): Calmodulin-binding drugs affect responses to cytokinin, auxin and gibberellic acid. Plant Physiol. **72**: 219–224.
- Elliott, D. C., Skinner, J. D. (1986): Calcium-dependent, phospholipid-activated protein kinase C type enzyme from plants. Phytochemistry **25**: 39–44.
- Elliott, D. C. (1989): Calcium and Diacylglycerol as signal in cytokinin-dependent pigment synthesis in Amaranthus seedling. In: Krekule, J., Seidlová, F. (eds): "Proceedings of Colloquia 32 and 33 of the 14th Biochemical Congress held in Prague", SPD Academic Publishing bv, The Hague, The Netherlands, pp.11–24.
- Ettlinger, C., Lehle, L. (1987): Auxin induces rapid changes in phosphatidylinositol metabolites. Nature **331**: 176–178.
- Goodwin, P. B., Gollnow, B. I., Letham, D. S. (1978): Phytohormones and growth correlations. In: Letham, D. S., Goodwin, P. B., Higgins, T. J. V. (eds): "Phytohormones and Related Compounds — A Comprehensive Treatise", Vol. II. Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 215–249.
- Kendra, D. V., Hadwiger, L. A. (1987): Calcium and calmodulin may not regulate the disease resistance and pisatin formation responses of Pisum sativum to chitosan or Fusarium solani. Physiol. Mol. Plant Pathol. **31**: 337–348.
- Keppler, L. O., Baker, C. J. (1989): O_2^- -initiated lipid peroxidation in a bacteria-induced hypersensitive reaction in tobacco cell suspensions. Phytopathology **79**: 555–562.

- Kikkawa, U., Kitano, T., Saito, N., Kishimoto, A., Taniyama, K., Tanaka, C., Nishizuka, Y. (1986): Role of protein kinase C in calcium-mediated signal transduction. In: Evered, D., Whelan, J. (eds): Calcium and the Cell. Ciba Foundation Symp. 122. John Wiley and Sons, Chichester, pp. 39–57.
- Kurosaki, F., Tsurusawa, Y., Nishi, A. (1987): Breakdown of phosphatidylinositol during the elicitation of phytoalexin production in cultured carrot cells. Plant Physiol. **85**: 601–604.
- Ladyzhenskaya, E. P., Korableva, N. P., Morozova, T. M., Sidorkina, O. M., Salgani, R. I. (1987): Activation of protein-kinase C in plant cells after treatment with gibberellic acid: translocation of the enzyme from the cytoplasm into the membrane fraction (in Russian). Fiziologija Rastenij **34**: 763–765.
- Marmé, D. (1983): Calcium transport and function. In: Läuchli, A., Bielsky, E. L. (eds): "Inorganic Plant Nutrition", Encyclopedia of Plant Physiology, New Series 158, . 599–625.
- Morse, M. J., Satter, R. L., Crain, R. C., Coté, G. G. (1989): Signal transduction and phosphatidylinositol turnover in plants. Physiol. Plant. **76**: 118–121.
- Oláh, Z., Bérczi, A., Erdei, L. (1983): Benzylaminopurine-induced coupling between calmodulin and Ca-ATPase in wheat root microsomal membranes. FEBS Lett. **154**: 395–399.
- Oláh, Z., Kiss, Z. (1986): Occurrence of lipid and phorbol ester activated protein kinase in wheat cells. FEBS Lett. **195**: 33–37.
- Sandelius, A. S., Sommarin, M. (1986): Phosphorylation of phosphatidylinositol in isolated membranes. FEBS Lett. **201**: 282–286.
- Strasser, H., Hoffmann, C., Grisebach, H., Matern, U. (1986): Are polyphosphoinositides involved in signal transduction of elicitor-induced phytoalexin synthesis in cultured plant cells? Z. Naturforsch. **41c**: 717–724.
- Weiss, B., Prozialeck, W., Cimino, M., Barnette, M. S., Wallace, T. L. (1980): Pharmacological regulation of calmodulin. Ann. N. Y. Acad. Sci. **356**: 319–345.

GROWTH AND ALKALOID CONTENT CHARACTERISTICS OF ISOLATED ROOTS OF DATURA INNOXIA MILL.

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Isolated root cultures of *Datura innoxia* were initiated. In attempt to achieve vigorous growth the isolated roots were tested for their response to an auxin supplementation. Gibberellic acid, known to stimulate alkaloid accumulation in *Datura* excised roots (GIBSON and FRENCH 1964), was additionally supplied to the nutrient medium containing auxin. The roots grown in the presence of auxin, auxin-gibberellin combination or in the absence of growth regulators substantially differed in their alkaloid content characteristics. The difference in hyoscyamine/scopolamine ratios was most probably due to the type of promoted growth.

Introduction

Tropane alkaloids constitute one distinct group of secondary metabolites produced mainly by the Solanaceae plants. Among these hyoscyamine and scopolamine are the principle alkaloids of medicinal interest. It has been found that the root is site of tropane alkaloid formation in many solanaceous plants (WALLER and NOVACKI 1978). Thus, the roots appear to be an interesting material for providing both the physiological and biosynthetic studies. In investigations carried out with isolated root cultures of solanaceous plants the main focus of interest was alkaloids.

The close relation between root organization and alkaloid synthesis in *Datura innoxia* has been recognized (HIRAOKA and TABATA 1974). Roots of the above-mentioned plant species were mainly used in biosynthetic studies (EVANS and GRIFFIN 1962; ROMEIKE 1971). However, there are no enough data

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about the growth characteristics of isolated roots of Datura innoxia if those are cultivated in vitro.

This work has been initiated in order to study the interactions between the type of growth and tropane alkaloid accumulation in isolated roots of Datura innoxia.

Material and Methods

Plant material

Seeds of Datura innoxia Mill. (botanical garden — Tries) were copiously washed in soapy water, soaked in 70% (v/v) ethanol for 1 min, immersed in 5% (w/v) calcium hypochlorite for 10 min and rinsed three times with sterile distilled water. Sterilized seeds were germinated on Murashige—Skoog's (MURASHIGE and SKOOG 1962) basic nutrient medium supplemented with 0.05 mg·l⁻¹ NAA (Naphthaleneacetic acid), 0.05 mg·l⁻¹ kinetin, 0.05 mg·l⁻¹ GA₃ (Gibberellic acid), 200 mg·l⁻¹ yeast extract, 200 mg·l⁻¹ casein hydrolysate, 200 mg·l⁻¹ myoinositol, 0.5% (w/v) D-glucose and 1.5% (w/v) sucrose. Resultant young seedlings served as a donor material for initiation of root cultures.

Initiation of root cultures

Primary roots (1 cm in length) were excised from donor seedlings and grown on solid Murashige—Skoog's basic medium, supplemented with 3% (w/v) sucrose. The following variants of growth regulator supplementation were used:

- I — MS basic (no growth regulators were added)
- II — MS basic + 0.5 mg·l⁻¹ IBA (Indole-3-butyric acid)
- III — MS basic + 1 mg·l⁻¹ IBA
- IV — MS basic + 0.5 mg·l⁻¹ IBA + 0.1 mg·l⁻¹ GA₃
- V — MS basic + 1 mg·l⁻¹ IBA + 0.1 mg·l⁻¹ GA₃

The roots were cultivated in Petri-dishes, containing 25 ml nutrient medium. The Petri-dishes were sealed with Parafilm and kept in the dark at 26 °C. Five replicate cultures were set up for each variant of growth regulator supplementation.

At the end of the cultivation period (20 days) roots fresh weights were determined.

Extraction and purification of alkaloids

Plant samples were freeze-dried, then powdered and extracted with NH₄OH — 10%/CHCl₃ (1:9) mixture in sonicating bath (TESLA Vrable, k.p.; UC 002 BM1) for 20 min. The extraction procedure was repeated three times and the combined extracts were evaporated to dryness. The dry residues were collected by washing with 2% H₂SO₄. The acidic-aqueous solutions were made alkaline (pH 8-9) by adding NH₄OH (20%) and extracted with CHCl₃. The chloroform fractions were dried by filtering through anhydrous Na₂SO₄ and evaporated to dryness. The residues dissolved in appropriate quantities of CHCl₃ were used for alkaloid determination.

Alkaloid detection and quantification

Alkaloid detection was performed by thin-layer chromatography. The TLC separation was carried out on silicagel 60 F₂₅₄ Merck plates. The chromatograms were developed by using 0.2 M NaAc/CH₃OH/CHCl₃/n-hexan (1:6:3:1) as a mobile phase, which was found to yield quite satisfactory result for tropane alkaloids (BOTZ and SZABÓ 1988). The alkaloid spots were located by Dragendorff's reagent (MUNIER and MACHEBOEUF 1951) in which the quantity of stock solution was changed to 3 ml. The TLC-densitometry was carried out by using a Shimadzu densitometer,

Table 1

Biomass production and alkaloid content in isolated roots of *Datura innoxia* Mill.
after 20 days cultivation

MS basic/ growth regulator supply	Fresh wt. (g) per culture		Alkaloid content (% of dry wt.)				Hyos./ Scopol. ratio
			Hyoscyamine		Scopolamine		
	Mean	<u>+SE</u> ^a	Mean	<u>+SE</u>	Mean	<u>+SE</u>	
—	0.4537	0.2675	0.0008	0.0010	0.0069	0.0012	0.12
0.5 mg·l ⁻¹ IBA	2.8130	2.3547	0.0062	0.0010	0.0083	0.0020	0.75
1 mg·l ⁻¹ IBA	2.3190	0.9258	0.0021	0.0012	0.0036	0.0014	0.58
0.5 mg·l ⁻¹ IBA	1.0250	0.3753	0.0011	0.0014	0.0031	0.0015	0.35
0.1 mg·l ⁻¹ GA ₃							
<hr/>							
Roots of donor plants — 20 days after root formation			0.0026	0.0015	0.0017	0.0018	1.53

^aStandard error

consisting of high speed dual-wavelength scanner CS-930 (Shimadzu, Japan) and DR-2 data recorder at 530 and 660 nm.

For quantitative and qualitative estimation of alkaloid compounds cochromatography with authentic standards (atropine sulfate and scopolamine hydrobromide — Reanal, Budapest) was carried out. Both atropine and scopolamine were calculated as base substances. In the present study, no distinction was made between *l*-hyoscyamine and atropine (*d,l*-hyoscyamine).

The alkaloid contents of the samples were calculated in per cent based on the dry weight of plant material. The experimental data of both biomass production and alkaloid content were statistically processed according to GORDON and FORD (1972).

Results and Discussion

Growth response

Cultivation of roots on nutrient medium without growth regulators did not lead to a satisfactory growth. The roots were tender and exhibited slight tendency for root hairs formation (Fig. 1). Lateral branching was not observed in those cultures. The growth was ensured by the tip meristem of the main root only. The slow growth presented in those cultures did not ensure high biomass production at the end of the cultivation period (Table 1).

The presence of 0.5 mg·l⁻¹ IBA affected positively the growth isolated roots. The elongation of the main root was accompanied by generation of several lateral meristematic points. The resultant lateral roots exhibited tendency for secondary branching (Fig. 2). Extensive profusion of root hairs



Fig. 1. Roots grown on nutrient medium free of growth regulators

was observed in those cultures. At the end of the cultivation period the older parts of the main root turned thick. The extensive lateral branching expressed in those cultures led to relatively high biomass production (Table 1).

Growth promoted by higher auxin supply ($1 \text{ mg} \cdot \text{l}^{-1}$ IBA) was rather weak. Lateral branching took place in the cultures, but it was not so vigorous as in roots grown on lower IBA concentration. The older parts of the roots turned light brown after 10 days of cultivation. The process of ageing was accompanied by callus formation presented mainly on the initial explant parts (Fig. 3).

Roots cultivated on a nutrient medium containing both IBA and GA_3 did not exhibit growth characteristics similar to those of cultures grown on nutrient media without gibberellic acid. Additional supplementation of GA_3 to the lower auxin concentration resulted in rather weak lateral branching. Lateral roots were tender and did not show extensive growth (Fig. 4). On various points along the main root callus formations were observed. Gibberellic acid, added in the same concentration to the higher auxin supply failed to provoke organized growth.

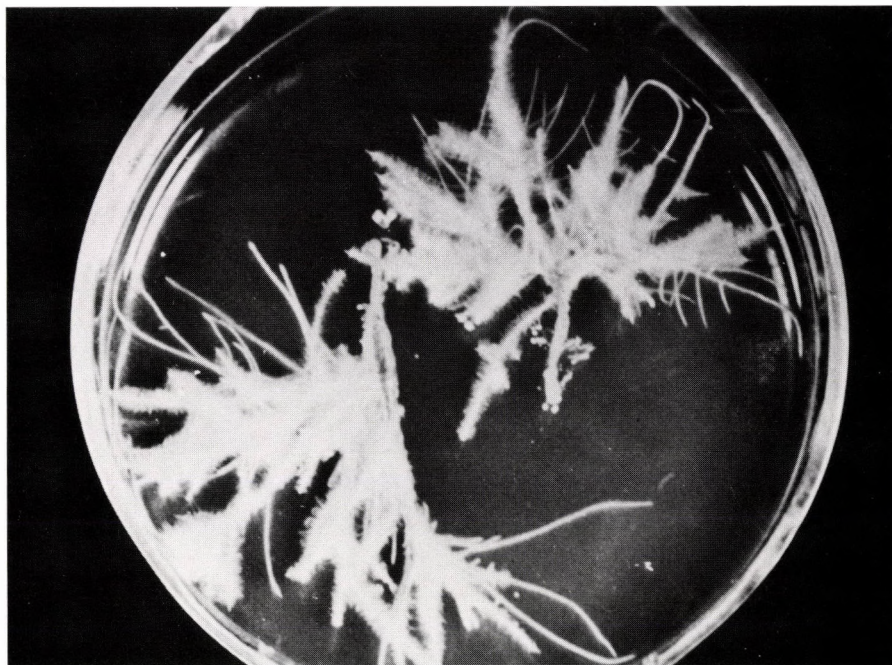


Fig. 2. Roots grown on nutrient medium, supplemented with $0.5 \text{ mg} \cdot \text{l}^{-1}$ IBA

Alkaloid content characteristics

Isolated roots significantly differed in their alkaloid content characteristics from roots of donor plants. Scopolamine was predominant in root cultures, whereas in roots of parent plants the leading position was taken by hyoscyamine (Table 1). Although hyoscyamine/scopolamine ratios of all cultures were strongly diminished, roots grown without or in presence of growth regulators differed substantially in their major alkaloids ratios. The lowest hyoscyamine/scopolamine ratio was observed in roots grown on nutrient medium free of growth regulators. Hyoscyamine content of those roots was approximately eight times lower than that of scopolamine. The highest hyoscyamine/scopolamine ratio was observed in roots cultured on $0.5 \text{ mg} \cdot \text{l}^{-1}$ IBA. Hyoscyamine production of those cultures was eight times higher than that of roots grown on phytohormone-free medium, whereas the difference in scopolamine production was not so substantial. Gibberellic acid in combination with an auxin reduced the production of both hyoscyamine and scopolamine. However, hyoscyamine production of roots was more considerably affected. In cultures grown on higher auxin supply the content of both hvo-

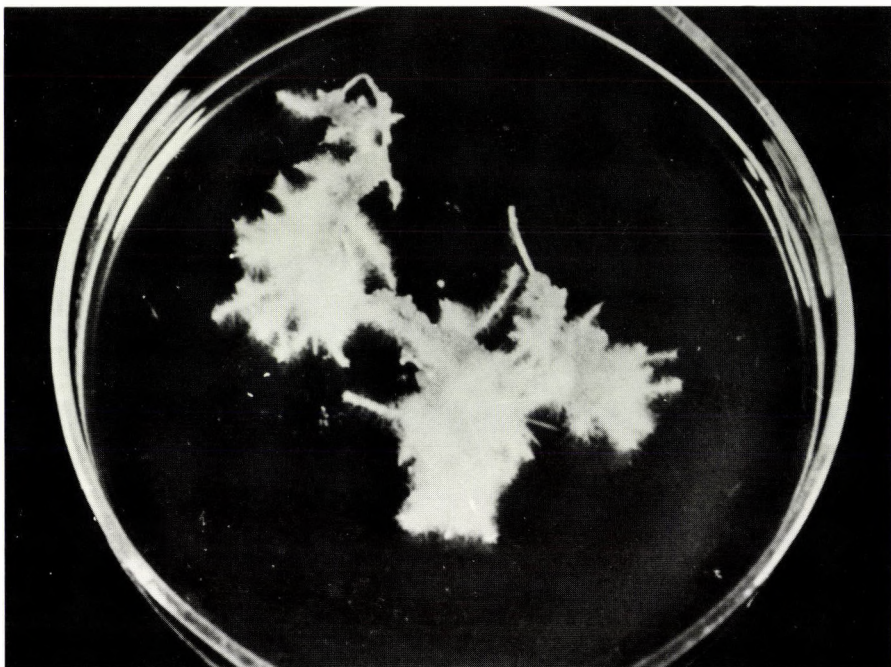


Fig. 3. Isolated roots cultivated on nutrient medium, supplemented with $1 \text{ mg} \cdot \text{l}^{-1}$ IBA

scyamine and scopolamine was approximately three times lower than that of roots cultured on the lower auxin supplementation.

Auxins are required to stimulate the growth of excised roots (SKOOG and MILLER 1957). However, in cultures of *Datura innoxia* better stimulation effect was observed if the auxin had been supplied in low concentration. Similar response to auxin supplementation was already recognized for cultures of several dycotyledonous plants (BUTCHER and STREET 1964).

Gibberellic acid alone does not inhibit growth of *Datura* excised roots (GIBSON and FRENCH 1964), but if supplied to nutrient medium containing IBA, it strongly diminished its promotory effect. These results indicate that GA_3 suppresses the positive effect of IBA.

The appreciable difference in alkaloid content characteristics of isolated roots cultivated in the presence of auxin, auxin-gibberellin combination or in the absence of growth regulators seems to be due to different type of growth promoted. Higher hyoscyamine production was observed in roots whose growth was accompanied by generation of several growing points. Thus the tested phytohormones affected indirectly the secondary product accumulation.

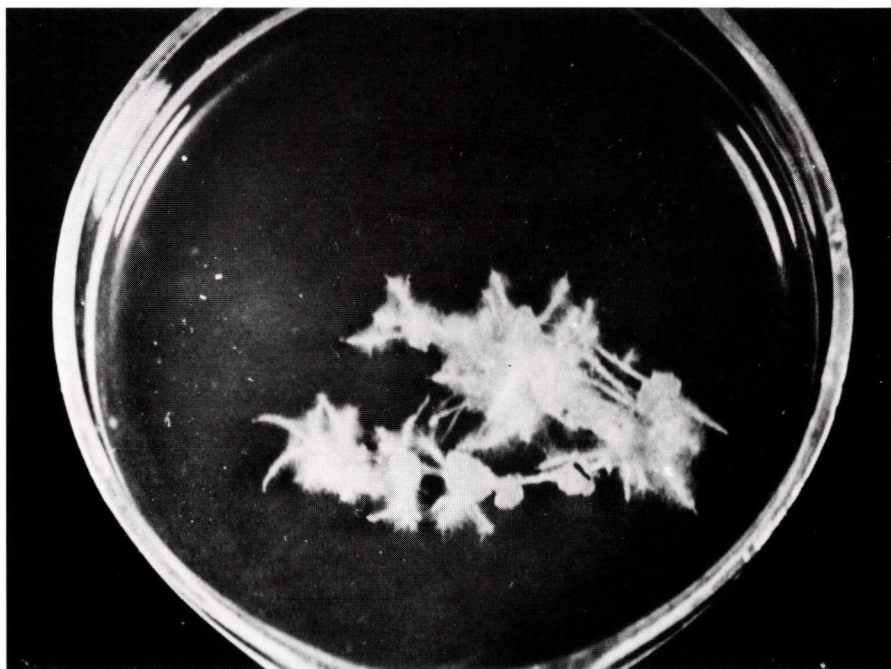


Fig. 4. Roots grown on nutrient medium, supplemented with $0.5 \text{ mg} \cdot \text{l}^{-1}$ IBA and $0.1 \text{ mg} \cdot \text{l}^{-1}$ GA₃

The results concerning the difference in the hyoscyamine content indicate that the process of the formation of the tropane ring including its esterification are closely related to an actively growing root tissue. The close relationship between the root growth with respect to the number of growing points and the alkaloid production suggests the existence of activities in the root tips. In this connection it should be mentioned, that similar conclusions were reached in the case of nicotine synthesis in excised roots of tobacco (SOLT 1957).

In all cultures the scopolamine was predominant which indicates that the root tissues have an enzymatic system developed to convert hyoscyamine into scopolamine. In this connection it should be mentioned that ROMEIKE (1971) reported on the ability of excised roots of *Datura innoxia* to carry out epoxidation if those are fed with nutrient solution containing hyoscyamine. Our findings revealed that in the case of *Datura innoxia* the development of whole plant is not necessary to ensure scopolamine synthesis.

REFERENCES

- Botz, L., Szabó, L. (1988): Separation of tropane alkaloids by TLC, HPTLC and OPLC methods. J. Planar Chrom. 1: 85—87.
- Butcher, D. N., Street, H. E. (1964): Excised root culture. Bot. Rev. 30: 513—586.
- Evans, W. C., Griffin, W. J. (1962): Production of alkaloids in the root of Datura innoxia under various conditions of culture. Lloydia 25: 139—141.
- Gibson, M. R., French, D. I. (1964): The effect of gibberellic acid on growth and alkaloid synthesis in Datura stramonium var. tatula. Lloydia 27: 47—52.
- Gordon, A. J., Ford, R. A. (1972): The Chemist's Companion. VIII. 512—524.
- Hiraoka, N., Tabata, M. (1974): Alkaloid production by plants regenerated from cultured cells of Datura innoxia. Phytochem. 13: 1671—1675.
- Munier, R., Macheboeuf, M. (1951): Bull. Soc. Chim. Biol. 33: 864.
- Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15: 473—497.
- Romeike, A. (1971): Untersuchungen über den Alkaloidstoffwechsel in Datura Wurzeln. IV. Beobachtung an Wurzelkulturen von Datura innoxia Mill. nach exogener "Zufuhr" von Atropin. Biochem. Physiol. Pflanzen 162: 1—8.
- Skoog, F., Miller, C. O. (1957): Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. Exp. Biol. 11: 118.
- Solt, M. L. (1957): Nicotine production and growth of excised tobacco root cultures. Plant Physiol. 32: 480—484.
- Waller, G. R., Novacki, E. K. (1978): Alkaloid Biology and Metabolism in Plants. Plenum Press, N. Y., London.

PROLINE CONCENTRATION, DROUGHT TOLERANCE AND WATER DEFICIENCY IN WHEAT VARIETIES

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The degree of drought tolerance was determined for 12 wheat varieties in the phase of flowering, on the basis of the response of proline concentration to the live wilting of isolated leaves and spikes. With the new method of live wilting identical level — lethal — internal water deficiency was achieved in 3 days with the leaves of each variety. Three of the varieties showed outstanding increase in proline concentration (145.2-136.1-133.0%). With the isolated spikes a result rather similar to that of the leaves was obtained. Proline accumulation in spikes was, however, essentially lower than in leaves (1/3 or 1/4 of it). In the case of 44 cultivated species two groups could be formed on the basis of their ability to accumulate proline: 1) species producing very large amounts of proline (between 1.0 and 4.0%); 2) those accumulating lower amounts of proline (below 1.0%). The proline accumulating ability of species is not linked with such taxonomical units as dicotyledons, monocotyledons or families, but species belonging to the same genus showed the same ability of proline accumulation. The proline accumulation ability of species belonging to different genera does not express the degree of drought tolerance compared to one another. Therefore the proline test as a method for determining drought tolerance can only be used when comparing improved varieties of the same species.

Keywords: winter wheat varieties, plant families, proline, total amino acid, isolated leaves, live wilting, lethal water deficiency, pollen viability

Introduction

It has been established that in response to such a water deficiency stress as caused by the arid, frosty or salty soil, the free proline accumulates to a great extent in the herbaceous mesophytic plants (PÁLFI and JUHÁSZ 1970; BLUM and EBERCON 1976; MALI and MEHTA 1977; LEWITT 1980; SIMI-NOVITCH and CLOUTIER 1981; VAN SWAAIJ *et al.* 1985; SIVARAMAKRISHNAN *et al.* 1988). Proline was found to play an important role in increasing the osmotic pressure of tissues, and the water retention ability, respectively, representing an amino nitrogen reserve and storing energy (SINGH *et al.* 1972; PÁLFI *et al.* 1974; HUBAC 1980; PALEG and ASPINALL 1981). Further advantages

of the proline are that of all amino acids it dissolves by far the best in water, inhibits least even at high concentrations the growth and development of tissues, and is the most stable amino acid (PÁLFI et al. 1974, 1975; LEWITT 1980; PALEG and ASPINALL 1981; PÁLFI et al. 1983; VAN SWAAIJ et al. 1985).

As it has been pointed out, the species cannot be compared for drought tolerance on the basis of proline accumulation ability (WALDREN and TEARE 1974; PÁLFI et al. 1975; PATEL and VORA 1985). On the other hand, we have found in agreement with others' statement that among improved varieties of the same species the one that accumulates more proline in the leaves in response to the same level of extreme water deficiency is more tolerant to drought (PÁLFI 1971; SINGH et al. 1972; BLUM and EBERCON 1976; MALI and MEHTA 1977; SASHIDHAR et al. 1977; SRINIVASA 1977; PÁLFI et al. 1978; PALEG and ASPINALL 1981; VAN SWAAIJ et al. 1985; SIVARAMAKRISHNAN et al. 1988). It follows that by proline analysis performed after a high water deficiency the degree of drought tolerance can be determined for varieties belonging to the same species.

According to VAN DE DIJK (1981) "external water deficiency" of the same measure may induce different degrees of "internal water deficiency" in the leaves of varieties belonging to the same species. Considering that this is the very feature in which the varieties differ from one another as regards drought tolerance, the internal water deficiency of the leaves must be identical if the drought tolerance of different varieties is to be determined.

In the present experiment we tried to so provoke a gradually developing water deficiency in leaves of 12 winter wheat varieties isolated at the time of flowering as to finally achieve the same degree of internal water deficiency in them. Then we determined the proline concentration of these water deficient leaves. Furthermore, by provoking a severe water deficiency in isolated leaves or removed shoots of plant species belonging to various families and genera we studied their proline accumulation ability and total amino acid content. We examined what taxonomic category the extent of proline accumulation was linked with: whether it was a character of dicotyledons, monocotyledons, families or genera, or an exclusive specific feature.

Material and Method

Breeding and maintenance of the wheat varieties were carried on the grounds of the Cereal Research Institute, Szeged. The names of the varieties are given in tables. The plants belonging to various species were taken from the Botanical Garden of the University. Consider-

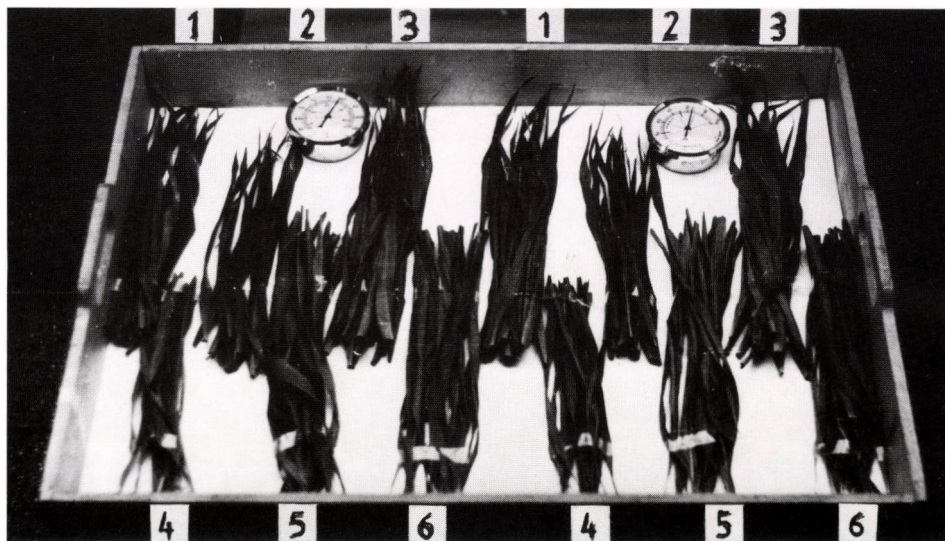


Fig. 1. Provoking lethal water deficiency by 3-day live wilting in wheat leaves isolated at the time of flowering. The 40 leaves removed from each of the 6 varieties were spread and fixed in 2 groups per variety, in order to achieve a steady intensity of illumination. The varieties were: 1 = GK Űthalom; 2 = GK Zombor; 3 = GK Kincs ; 4 = GK S gv ri; 5 = GK Kalangya; 6 = GK Bence

ing that in Hungary the critical water deficiency of wheat mostly occurs in the development phase following flowering, the leaf samples were taken when the first anthers appearing in the middle of the spike dehisced.

In consequence of the hot weather in the year of the investigation the varieties of the 3 maturity groups of wheat completed flowering in 4 days. We therefore formed two groups of the early-, medium-early- and medium-late-varieties, with 6 varieties in each.



Fig. 2. Provoking lethal water deficiency by 3-day live wilting in spikes isolated at the time of flowering. The order of variety is the same as in Fig. 1

For the purpose of provoking water deficiency the upper two leaves of 20 shoots per variety were cut off. They were perfectly intact leaves free of lesions. The isolated leaves were fixed in two groups per variety on a plate covered with filter paper, in order to ensure uniform distribution of light (Fig. 1).

To achieve extreme water deficiency the phytotron was adjusted in the following way: 26-28 °C temperature, 90% humidity and 5000 lux constant illumination for 60 hours. Finally, for further 12 hours humidity was reduced to 60% in order to attain the lethal water deficiency of leaves for all varieties, that is, a uniform "internal water deficiency" of leaves.

The above described method of developing lethal water deficiency in 3 days will hereinafter be called "live wilting".

After live wilting the leaves were cut into pieces, dried at 90 °C, then reduced to powder. The leaf powders were then stabilized, hermetically sealed and stored. The spikes of the same shoots were cut off and similarly subjected to the 3-day procedure of live wilting (Fig. 2).

The proline analysis of the pulverized leaves and spikes was performed by the method of PALEG and ASPINALL (1981); the total amino acid contents were determined after ROSEN (1957). Live wilting and proline analysis were also carried out with shoots or leaves isolated at the time of flowering from 44 plant species belonging to 11 families of dicotyledons and monocotyledons. With this we tried to determine the proline accumulation ability of the different species.

Results and Discussion

By weighing the isolated shoots and leaves we found that they lost suddenly or in the first 24 hours much of their normal water content (35-40 per cent), if the air was not saturated with vapour. During that time the total amino acid content considerably increased, while proline only began to accumulate afterwards, as a consequence of a high water deficiency. This phenomenon was published by WALDREN and TEARE (1974) and LEVITT (1980) too.

Table 1 gives the proline concentrations of live wilted wheat leaves for each variety examined.

As seen in Table 1 out of the 6 early wheats the prospective variety "GK Kalangya" has an outstanding proline concentration in the leaves (3.02%). This proline content means a 45.2 per cent increase compared to "GK Ságvári", the variety accumulating the lowest amount of proline. Among the early wheats "GK Öthalom" and "GK Kincső" also deserve attention with their live wilting results of 16.3 and 12.5 per cent proline increase.

In the group of medium early varieties (Table 1) the lowest proline concentration was pointed out for "GK Örzse" (100%). In comparison to this the increase in proline content was outstanding in two varieties: "GK Szőke" and "Jubilejnaja 50" (36.1 and 33.0%, respectively). Another variety that showed a considerable proline increase in this group was "GK István" (12.9%).

The method of provoking lethal water deficiency by live wilting the isolated leaves may reconcile the controversy between those who have elabo-

Table 1

Proline concentration developing in response to 3 days of live wilting in leaves of 12 wheat varieties isolated at the time of flowering. The proline contents are also given as a percentage of the lowest proline concentration variety

Number	Name of wheat cultivars	Proline concentration in per cent of dry matter	of control
early ripenings			
1.	GK Őthalom	2.42 ± 0.11	116.3
2.	GK Zombor	2.27 ± 0.10	109.1
3.	GK Kincső	2.34 ± 0.10	112.5
4.	GK Ságvári	2.08 ± 0.09	100.0
5.	GK Kalangya	3.02 ± 0.14	145.2
6.	GK Bence	2.15 ± 0.10	103.4
middle ripenings			
7.	Bucsányi 20	2.55 ± 0.12	109.4
8.	GK István	2.63 ± 0.11	112.9
9.	GK Szőke	3.17 ± 0.15	136.1
10.	Jubelejnjaja 50	3.10 ± 0.14	133.0
11.	SGV. — F 29-76	2.47 ± 0.11	106.0
12.	GK Őrzse	2.33 ± 0.10	100.0

In the repetitions of the analyses the standard deviation of the mean error is within $\pm 5\%$; $n = 3$

rated and employ the proline test of drought tolerance (SINGH *et al.* 1972; PÁLFI *et al.* 1974, 1975; BLUM and EBERCON 1976; MALI and MEHTA 1977; PALEG and ASPINALL 1981; SIMINOVITCH and CLOUTIER 1981; VAN DE DIJK 1981; VAN SWAAIJ *et al.* 1985; SIVARAMAKRISHNAN *et al.* 1988) and those who disapprove of the method (GUPTA and SHEORAN 1979; HANSON *et al.* 1977, 1979; ILAHI and DÖRFFLING 1982). Latter, i.e. the opposers of the method, reported on having attained different levels of "internal water deficiency" in still living leaves beside a uniform level of "external water deficiency" in the course of provoking water deficiency mostly in young plants (namely, this is the very point in which the varieties differ from one another while accomodate themselves to drought!).

With our new method of live wilting every leaf sample taken at the time of flowering attains the same "lethal internal water deficiency".

Table 2

Proline concentrations in response to lethal water deficiency in spikes isolated at the time of flowering and live wilted for 3 days

Number	Name of wheat cultivars	Proline concentration in per cent of dry matter	of control
early ripenings			
1.	GK Őthalom	0.75 ± 0.03	144.2
2.	GK Zombor	0.66 ± 0.03	126.9
3.	GK Kincső	0.91 ± 0.04	175.0
4.	GK Ságvári	0.52 ± 0.02	100.0
5.	GK Kalangya	0.76 ± 0.04	146.1
6.	GK Bence	0.59 ± 0.03	113.5
middle ripenings			
7.	Bucsányi 200	0.94 ± 0.04	154.1
8.	GK István	0.65 ± 0.03	106.5
9.	GK Szőke	1.15 ± 0.05	188.5
10.	Jubilejnaja 50	1.19 ± 0.05	195.1
11.	SGV. — F 29-76	0.86 ± 0.04	141.0
12.	GK Őrzse	0.61 ± 0.03	100.0

In the repetitions of the analyses the standard deviation of the mean error is within $\pm 5\%$; $n = 3$

The concentrations of proline accumulated in consequence of live wilting isolated spikes of the wheat varieties are shown in Table 2.

As seen in Table 2 proline accumulation in the isolated spikes is only about one-quarter to one-fifth of that in the leaves; which is quite natural, as the chloroplast content of spikes is only a fraction of that of leaves, and the intensity of photosynthesis — and of light, respectively — plays an important role in proline formation, considering that this reaction requires carbohydrate, ATP and NADH_2 (LEVITT 1980; PALEG and ASPINALL 1981).

Among the early varieties the highest proline concentration in response to lethal water deficiency was obtained with "GK Kincső" (175.0%); very high proline levels were attained by "GK Őthalom" and "GK Kalangya" too (144.2 and 146.1%, respectively). The lowest proline content in spikes — just like in leaves — was found in "GK Ságvári" (100%). This result is somewhat similar to, but not identical with the order of proline content

obtained with the leaves. The isolated spikes of the medium early wheat varieties, on the other hand, showed a quite similar order of proline accumulation as the leaves. The proline concentration of spikes was similarly the lowest (100%) in "GK Őrzse", and the highest in "GK Szőke" and "Jubilejnaja 50" (188.5 and 195.1%, respectively). In any case it can be taken into consideration that the varieties do not differ in the chloroplast content of leaves so much as seen with the spikes, therefore the live wilting of leaves, and their proline test, respectively, may give a more reliable result.

If the degree of drought tolerance demonstrated by live wilting at the time of flowering proves valid in practice, the variety accumulating more proline in the case of water deficiency must produce a higher grain yield in the field. In the year of the experiment (1988) extremely hot, rainless weather prevailed from flowering up to the time of harvest. The grain crop was choked, its quantity was considerably reduced all over Hungary compared to what had been expected.

In the early group it was for the prospective variety "GK Kalangya" that the highest proline accumulation was pointed out. The National Institute for Agricultural Qualification carried out small-plot experiments with 38 prospective varieties in 6 replications on 21 different areas of Hungary (CZIRÁK 1988). Out of the 38 prospective varieties "GK Kalangya", the one preferred by us gave the largest volume of yield on the average of 21 replications in the year with a dry period at the end of vegetation (7960 kg/ha). In the same year HARMATI (1988) carried out large plot yield experiments with registered varieties. On meadow soil and sand out of the 12 medium early varieties "Jubilejnaja 50" gave the third largest yield (8.65 t/ha). This variety is known to be a standard providing medium to good yield even under extreme conditions. In large-plot experiments of the same year out of 4 medium late varieties "GK Szőke" produced the largest grain yield both on meadow soil and on sand (11.15 and 9.40 t/ha, respectively), and this variety showed the highest proline content too.

As it is seen, the degrees of drought tolerance established in advance by proline test at the time of flowering in a water deficient cropyear were in agreement with the yield in the field.

Proline accumulation ability developed in response to lethal water deficiency in leaves or shoots isolated at the time of flowering is shown for plant species of 11 families in Table 3.

Table 3

Ability of various plant species to accumulate proline in response to lethal water deficiency in leaves isolated at the time of flowering. Live wilting took 3 days. (The species of the different families are separated by a horizontal line.) For species with very small leaves, such as *Medicago*, *Trifolium* etc. live wilting was carried out with whole isolated shoots. The concentrations are given in terms of dry matter percentage

Species	Proline per cent	Total amino acid	Species	Proline per cent	Total amino acid
<u>Trifolium repens</u>	2.37	8.63	<u>Papaver somniferum</u>	0.41	6.52
<u>Phaesus vulgaris</u>	0.56	6.81	<u>P. rhoas</u>	0.36	7.27
<u>Pisum sativum</u>	2.83	8.25	<u>Lactuca sativa</u>	0.27	7.82
<u>Lens culinaris</u>	1.45	7.54	<u>Artemisia vulgaris</u>	2.32	6.74
<u>Medicago sativa</u>	3.18	9.87	<u>Helianthus annuus</u>	2.25	6.77
<u>M. lupulina</u>	2.84	8.62	<u>Spinacia oleracea</u>	0.39	7.63
<u>Vitis vinifera</u>	0.56	6.88	<u>Beta vulgaris</u>	0.42	7.81
<u>Anethum graveolens</u>	1.73	6.74	<u>Rumex acetosa</u>	0.27	6.65
<u>Solanum lycopersicum</u>	1.75	7.65	<u>R. scutatus</u>	0.32	6.72
<u>S. tuberosum</u>	1.87	7.81	<u>Lolium perenne</u>	2.63	8.67
<u>S. laciniatum</u>	3.45	9.76	<u>L. aristatum</u>	2.56	7.82
<u>Capsicum annuum</u>	2.62	8.78	<u>Festuca pratensis</u>	1.75	7.63
<u>Nicotiana tabacum</u>	3.08	10.64	<u>F. vaginata</u>	1.82	7.06
<u>Hyoscyamus niger</u>	2.13	7.19	<u>Triticum aestivum</u>	3.14	9.84
<u>Raphanus sativum</u>	2.07	7.13	<u>T. durum</u>	2.87	8.68
<u>Sinapis alba</u>	1.82	6.64	<u>Hordeum vulgare</u>	1.78	7.56
<u>Brassica oleracea</u>	3.24	10.75	<u>H. hexastichon</u>	1.86	7.87
<u>B. napus</u>	2.38	8.68	<u>Secale cereale</u>	1.64	6.94
<u>Cucumis sativus</u>	0.42	6.63	<u>Avena sativa</u>	2.25	7.15
<u>C. melo</u>	0.35	6.85	<u>Sorghum vulgare</u>	0.47	8.26
<u>Cucurbita pepo</u>	0.26	7.04	<u>Zea mays</u>	0.42	8.58
<u>C. maxima</u>	0.43	6.58			
<u>Colocynthis citrullus</u>	0.34	6.79			

In the repetitions of the analyses the standard deviation of the mean error is within $\pm 5\%$; $n = 3$

On the basis of proline concentration the 44 species examined can be placed in two groups: in the leaves of group 1 the proline accumulation provoked by lethal water deficiency is extremely high ranging from 1.0 to 4.0 per cent. In the species of group 2 the proline content is much lower than in group 1, less than 1.0 per cent, although in comparison to the control, which was supplied with optimum amount of water, even these species

had a proline content of 300-500 per cent (PÁLFI et al. 1974, 1975, 1983). However, in group 1 the proline accumulation may even reach 5 to 10 thousand per cent.

Also, Table 3 shows that the proline accumulation ability does not express the degree of drought tolerance of species compared to one another. For example, in consequence of live wilting Triticum aestivum accumulated 3.14%, Zea mays only 0.42% proline, though as regards drought tolerance the latter is superior to the former.

Table 3 reveals that both among dicotyledons and monocotyledons as well as in the different families some species accumulate very large, other smaller amounts of proline. That is, the proline accumulation ability is independent from these taxonomic categories. On the other hand, there are 2 or 3 species in 11 genera in Table 3 each of which can be ranked either with species accumulating very much proline or with those accumulating small amounts of it. This suggests that proline accumulation ability is a generic characteristic. In any case it is sure that each variety of a culture species belongs to the same proline accumulation group. As it has been proved for the following species: Sorghum vulgare, Zea mays, Lactuca sativa, Spinacea oleracea, Cucumis sativus, Triticum aestivum, Secale cereale, Medicago sativa (PÁLFI et al. 1974, 1975, 1978, 1983; GULYÁS and PÁLFI 1986).

It is clear from Table 3 that the plants respond to the lethal water deficiency with a very high total amino acid concentration (6-11%). This suggests that a severe water deficiency of isolated leaves results in a considerable increase in the concentration of not only proline but also of other amino acids. In the case of optimum water supply the total amino acid is only 1.0 to 2.0% of the dry matter weight (PÁLFI et al. 1975, 1978, 1983).

Between changes in the total amino acid content and the degree of drought tolerance no correlation was found (PÁLFI et al. 1978, 1983; PÁLFI and PINTÉR 1980).

The proline concentration found in leaves of intact plants grown in the field following a severe dryness of soil is quite low compared to that in isolated live wilted leaves, generally not higher than 0.3-0.5% (PÁLFI 1971; PÁLFI et al. 1974, 1975, 1978). Namely, in isolated leaves replacement of water stops immediately after they have been cut off. In the case of field crops, on the other hand, from roots penetrating deeper some water flows even when the soil is extremely dry. "Lethal water deficiency" in field crops occurs therefore very seldom in Hungary, though the droughty weather may sharply reduce the volume of yield.

The proline data and proline test published here exclusively apply to isolated leaves, just as most works on similar subjects apply to isolated shoots, leaves or parts of them, and to plants grown in culture pots (SINGH et al. 1972; BLUM and EBERCON 1976; MALI and MEHTA 1977; GUPTA and SHEORAN 1979; HANSON et al. 1979; SIMINOVITCH and CLOUTIER 1981; SIVARAMAKRISHNAN et al. 1988; THAKUR et al. 1988).

It may also be taken into consideration that the proline accumulation ability is an evolutionary, hereditary feature (BLUM and EBERCON 1976; LEVITT 1980; PALEG and ASPINALL 1981; VAN SWAAIJ et al. 1985). Thus, this characteristic of the species and their cultivars may supposedly be transmitted by crossing or gene technology to high yielding but less drought tolerant varieties (WYN JONES and CORHAM 1986). By crossing related inbred maize lines this has partly been achieved (PINTÉR et al. 1981).

Numerous plant species have been reported to have very high proline concentrations (TUPY 1963; DASHEK and HARWOOD 1974; LINSKENS 1974; AHOKAS 1978; PÁLFI et al. 1981; GULYÁS and PÁLFI 1986; PÁLFI et al. 1987). In the course of studying 167 plant species we have found that the pollens of species can also be placed in two groups on the basis of their proline content:

- 1) pollens with very high (1.0 to 2.5%) proline content and

- 2) those with low (below 0.03%) proline content (PÁLFI and KÖVES 1984; PÁLFI and MIHALIK 1985; GULYÁS and PÁLFI 1986; PÁLFI et al. 1987). Most of the cultivated species accumulate very large amounts of proline in their pollens. The extent of proline accumulation is a characteristic of genera and species in the case of pollen too. Within a species those varieties which contain more proline in the pollen are of better quality (TUPY 1963; STANLEY and LINSKENS 1974; PÁLFI and KÖVES 1984; GULYÁS and PÁLFI 1986; PÁLFI et al. 1987). Proline plays the same role in the pollen as in the leaves: it gives protection against the stress of extreme weather conditions, in cold and frosty, or dry and hot weather (AHOKAS 1978; DASHEK and MILLS 1981; ZHANG et al. 1982; ZHANG and CROES 1983).

In an interesting way, the species in the two groups of leaves and pollens, that is those in the very high- and the low proline concentration group, respectively, are not always the same. For example, in the case of a lethal water deficiency the leaves of maize and sorghum belong to the low proline concentration group, while their mature pollen can be placed in the very high proline concentration group. And this occurs in the other way round too: the leaf of sunflower is able to accumulate a very large amount of proline in response to water deficiency, while its pollen only stores a

small amount of it. To determine the proline content of pollen and its quality, respectively, we have elaborated a new quick isatin staining method (PÁLFI and MIHALIK 1985; GULYÁS and PÁLFI 1986; PÁLFI et al. 1987).

REFERENCES

- Ahokas, H. (1978): Cytoplasmic male sterility of barley. II. Physiology and anther cytology. Hereditas **89**: 1–21.
- Blum, A., Ebercon, A. (1976): Genotypic responses in Sorghum to drought stress. III. Free proline accumulation and drought resistance. Crop Sci. **16**: 428–431.
- Czirák, L. (1988): Őszibúzafajta-összehasonlító kísérletek (Comparative studies of winter wheat cultivars). Magyar Mezőgazdaság **48**: 8–9.
- Dashek, W. V., Harwood, H. I. (1974): Proline, hydroxyproline and lily pollen tube elongation. Ann. Bot. **38**: 947–959.
- Dashek, W. V., Mills, R. R. (1981): Proline metabolism by germinating Lilium longiflorum. I. Labelling of cytoplasmic wall and culture medium molecule. Acta Soc. Bot. Pol. **50**: 51–56.
- Gulyás, S., Pálfi, G. (1986): Proline type pollens and their vitality in the Rosaceae and the species of other families. Acta Biol. Szeged **32**: 9–18.
- Gupta, P., Sheoran, I. S. (1979): Effect of water stress on the enzymes of nitrate metabolism in two Brassica species. Phytochemistry **18**: 1881–1882.
- Hanson, A. D., Nelson, C. E., Everson, E. H. (1977): Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. Crop Sci. **17**: 720–726.
- Hanson, A. D., Nelson, C. E., Pedersen, A. R., Everson, E. H. (1979): Capacity for proline accumulation during water stress in barley and its implications for breeding for drought resistance. Crop Sci. **19**: 489–493.
- Harmati, I. (1988): Búzafajta-kísérletek homokon és réti talajon (Experiments with wheat cultivars on sand and meadow soil). Magyar Mezőgazdaság **42**: 9.
- Hubac, H., Vieira Da Silva, J. (1980): Indicateurs métaboliques de contraintes mésologiques. Physiol. Veg. **18**: 45–53.
- Ilahi, I., Dörffling, K. (1982): Changes in abscisic acid and proline levels in maize varieties of different drought resistance. Physiol. Plant. **55**: 129–135.
- Levitt, J. (1980): Responses of plants to environmental stresses. II. Water, radiation, salt and other stresses. Acad. Press. N. Y., London, Sydney, San Francisco. 58 pp.
- Linskens, H. F. (1974): Fertilization in higher plants. North-Holland Publ. Co. Amsterdam. 37 pp.
- Mali, P. C., Mehta, S. L. (1977): Effect of drought on enzymes and free proline in rice varieties. Phytochemistry **16**: 1355–1357.
- Paleg, L. G., Aspinall, D. (1981): The Physiology and biochemistry of drought resistance in plants. Acad. Press. Sydney, N. Y., London, Toronto, San Francisco. 206 pp.
- Pálfi, G. (1971): The theoretical basis and practical application of a new method of selection for determining water deficiency in plants. Plant and Soil **34**: 503–507.
- Pálfi, G., Gulyás, S., Szöllősi I. (1987): Concentration of proline in pollens: a new taxonomical index. Acta Biol. Szeged **33**: 25–34.

- Pálfi, G., Juhász, J. (1970): Increase of the free proline level in water deficient leaves as a reaction to saline or cold root media. Acta Agron. Acad. Sci. Hung. 19: 79–88.
- Pálfi, G., Köves, E. (1984): Determination of vitality of pollen on the basis its amino acid content. Biochem. Physiol. Pflz. 179: 237–240.
- Pálfi, G., Köves, E., Bitó M., Sebestyén, R. (1974): The role of amino acids during water-stress in species accumulating proline. Fyton (Arg.) 32: 121–127.
- Pálfi, G., Köves, E., Nehéz, R. (1975): Effect of growth-substances on the free amino-acid content of lentil shoots in case of water deficiency. Acta Biol. Szeged 21: 69–82.
- Pálfi, G., Mihalik, E. (1985): Proline staining as a new method for determining the vitality of pollen grains in wind and insect pollinated plants. Acta Bot. Hung. 31: 315–321.
- Pálfi, G., Németh, J., Pintér, L., Kádár, K., Bölke, W. (1978): Rapid determination of drought-resistance of new rye, maize and lupine varieties with the live-wilting proline test. Acta Biol. Szeged 24: 39–51.
- Pálfi, G., Pálfi, Zs., Pintér, L. (1983): Enhancement of the degree of drought-resistance in varieties by crossing and selection on the basis of proline-content. Acta Biol. Szeged 29: 11–17.
- Pálfi, G., Pintér, L. (1980): Determination of the drought-resistance of inbred maize lines with proline test. Acta Biol. Szeged 26: 109–116.
- Pálfi, G., Pintér, L., Pálfi, Zs. (1981): The proline content and fertility of the pollen of inbred maize lines. Acta Biol. Acad. Sci. Hung. 27: 179–187.
- Patel, J., Vora, A. B. (1985): Free proline accumulation in drought-stressed plants. Plant and Soil. 84: 427–429.
- Pintér, L., Pálfi, G., Kálmán, L. (1981): Individual analyses of maize (*Zea mays* L.) plants for increased drought resistance. Z. Pflanzenzüchtg. 87: 260–263.
- Rosen, H. (1957): Modified colorimetric analysis of amino acids by ninyhydrine. Arch. of Biochem. and Biophys. 67: 10–15.
- Sashidhar, V. R., Meghri, A. A., Krishnaistry, K. S. (1977): Proline accumulation in relation to seed hardening in ground nut genotypes. Indian J. Agric. Sci. 47: 595–598.
- Siminovitch, D., Cloutier, Y. (1981): 24-Hour induction freezing and drought resistance in plumules of winter rye seedlings by desiccation at room temp. in the dark. Plant Physiol. Suppl. 67: 345.
- Singh, T. N., Paleg, L. G., Aspinall, D. (1972): Proline accumulation and varietal adaptability to drought in barley: a potential metabolic measure of drought resistance. Nature New Biology 236: 188–189.
- Sivaramakrishnan, S., Villoo, Z. Patell, Flower, D. J., Peacock, J. M. (1988): Proline accumulation and nitrate reductase activity in contrasting *Sorghum* lines during mid-season drought stress. Physiol. Plant. 74: 418–426.
- Srinivasa, V. (1977): Free proline accumulation and reduction RWC under moisture stress in genotypes safflower. Curr. Sci. Bangalore 46: 646–647.
- Stanley, R. G., Linskens, H. F. (1974): Pollen Biology, Biochemistry, Management. Springer Verl. Berlin, Heidelberg, N. Y. 67 pp.
- Thakur, P. S., Thakur, S., Rai, V. K. (1988): Reversal of stomatal closure *Commelina communis* L. by exogenous amino acids and their interaction with stress and abscisic acid. Biochem. Physiol. Pfl. 183: 37–43.
- Tupy, J. (1963): Free amino acids in apple pollen from the point of view of its fertility. Biol. Plant. 5: 154–164.

- Van de Dijk, S. J. (1981): Two ecologically distinct subspecies of Hypochaeris radicata L. III. Differences in drought resistances. Plant and Soil 63: 149-163.
- van Swaaij, A. C., Jacobsen, E., Feenstra, W. J. (1985): Effect of cold hardening, wilting and exogenously applied proline on leaf proline content and frost tolerance of several genotypes of Solanum. Physiol. Plant. 64: 230-236.
- Waldren, R. P., Teare, I. D. (1974): Free proline accumulation in drought stressed plants under laboratory conditions. Plant and Soil 40: 689-692.
- Wyn Jones, R. G., Corham, J. (1986): The potential for enhancing the salt tolerance of wheat and other important crop plants. Outlook Agric. Oxford 15: 33-39.
- Zhang, H. Q., Croes, A. F. (1983): Proline metabolism in pollen: degradation of proline during germination and early tube growth. Planta 159: 46-49.
- Zhang, H. Q., Croes, A. F., Linskens, H. F. (1982): Protein synthesis in germinating pollen of Petunia: role of proline. Planta 154: 199-203.

EFFECTS OF COPPER ON PROTEIN AND BIOMASS YIELDS OF RICE PLANTS

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Rice (*Oryza sativa* L.) plants were grown for one month in nutrient solutions containing copper concentrations ranging from 0.002 to 6.25 mg/l. The effects of increasing copper concentrations on protein content and shoot biomass were studied. In order to understand the mechanism responsible for the observed decline of protein content with increasing Cu levels, the membrane permeability was studied as well as the acid RNase and protease activities. It was found that the membrane permeability and the acid RNase activity increased with increasing copper concentration. An increase in the protease activity was also observed but only for the two highest copper treatments. The effects of increasing copper levels on protein and biomass changes are discussed on the basis of the measured acid RNase and protease activities.

Keywords: biomass yields, copper toxicity, rice, RNase, protease

Introduction

The pollution of our environment can no longer be stopped, only moderated (BELL 1980; DÄSSLER 1979; KOVÁCS, M. 1975, 1985). The production of rice in the Sado river (Portugal) it has become in the last years, a major problem due to Cu pollution from industry. It is therefore required to know how excess Cu affects the biomass production of rice. It has been reported (FERNANDES and HENRIQUES in press) that excess copper decreases protein content in plants. However, it is not known if excess copper affects mainly protein synthesis or proteolysis. Indeed, protein content of cells and tissues can be decreased either by an enhancement of acid RNase activity (DAVE and KANNAN 1980) or by uncontrolled protease activity (NEURATH 1984).

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It has been suggested that acid RNase is located in the cellular membranes (DAVE and KANNAN 1980) and that the enhancement of its activity indicates an alteration of the permeability of these membranes. The enzyme could degrade cytoplasmic or membrane bound RNA (DAVE and KANNAN 1980).

Proteolytic enzymes are ubiquitous in the cells and are activated either by limited proteolysis of inactive protease precursors (NEURATH 1984) or by release from complexes with protein inhibitors (NEURATH 1984). Protease inhibitors have been found in plants (NEURATH 1984) and it has been suggested that they act as pseudosubstrates by combining in an essentially irreversible manner with the active site of the enzyme (KOWALSKI *et al.* 1974).

This investigation presents the results of studies on protein content and biomass yields of rice plants submitted for 30 days to varying copper concentrations in the nutrient solution. These measurements are related to the acid RNase and protease activities as well as to variations of membrane permeability in order to identify the main mechanism(s) by which excess copper decrease(s) protein content in rice plants submitted to long-term excess copper treatments.

Material and Methods

Rice (*Oryza sativa* L. cv. Safari) seeds were washed in distilled water and sterilized by immersion in a mercury bichloride solution (1:1000) for 2 minutes. The seeds were then washed 5 times in deionized water and placed in an oven at 28 °C for 24 h. Seeds were germinated in moistened filter paper at 28 °C for 3 days. Seedlings were grown hydroponically for 30 days in cylindrical 21 pots at 35-37/25-27 °C day/night temperatures and under 250 $\mu\text{E PAR m}^{-2} \text{ s}^{-1}$ irradiance over a 12 h-day period.

The nutrient solution used was that of YOSHIDA *et al.* (1976) containing copper concentrations below normal (0.002 mg/l), normal (0.01 mg/l) and toxic (0.05; 0.25; 1.25 and 6.25 mg/l). Other nutrients were used at the following concentrations: N (40 mg/l); P (10 mg/l); K (40 mg/l); Ca (40 mg/l); Mg (40 mg/l); Mn (0.5 mg/l); Mo (0.05 mg/l); B (0.2 mg/l); Zn (0.01 mg/l); Fe (2 mg/l). The solution was adjusted to pH 5.5 daily and the volume brought to the original value with nutrient solution. The whole solution was renewed every 5 days.

The biomass yield was the average from the entire shoot fresh and dry weight of 100 rice plants. The dry weight was determined after placing the rice plants in an oven at 100 °C for 10 days.

Copper was measured from the entire root and shoot tissue of rice plants at the end of the 30 days experimental period, using a Perkin-Elmer model 3030 atomic absorption spectrophotometer, equipped with a hollow cathode lamp, after digestion of the samples in a nitric:perchloric (5:2, v/v) acid mixture followed by treatment with a nitric:sulfuric:perchloric (10:1:10 v/v/v) acid mixture (OHKI 1975).

Nitrogen was measured from the entire root and shoot tissue of rice plants at the end of the 30 days experimental period, using the regular macro-Kjeldahl method (BREMNER 1965).

Protein was determined according to the method of BRADFORD (1976), using a BSA standard curve. Shoot protein determination was based on subsamples of the entire shoot.

Electrical conductance of a solution arising from the incubation of rice shoots in water hereafter referred simply as electrolytic conductance was determined with a Crison 522 conductimeter using the modified method of KETCHIE (1969). Three grams of entire rice shoots were placed in an Erlenmeyer flask with 20 ml of deionized water for 8 hours and the absolute conductance measured. Then, the rice shoots were boiled for 7 minutes, the volume of deionized water was corrected and the totalelectrolytic yield obtained was taken as one hundred per cent. The results are expressed on a percent basis.

Protease activity was measured after enzyme extraction from an intimate mixture of entire shoots according to CHURCH *et al.* (1985), using subsamples and BSA as a substrate. The enzyme activity is expressed according to GOVIND *et al.* (1981) defining one unit of activity as the concentration in μ Moles of tryptophan released per ml of enzyme per hour, at 37 °C.

Acid RNase activity was assayed using subsamples after enzyme extraction from an intimate mixture of entire shoots according to DAVE and KANNAN (1980), by incubating the reaction mixture consisting of 0.2 ml each of acetate buffer (0.1 M pH 5.5), 0.15% purified high molecular weight RNA and crude enzyme extract in a waterbath at 37 °C for 30 min (SHORTMAN 1961). The reaction was terminated by the addition of 5.4 ml of alcoholic lanthanum nitrate reagent (AMBELLAN and HOLLAENDER 1966). Reaction tubes were kept in an icebath for 20 min, centrifuged at 2000 g for 30 min and the absorbance of the supernatant recorded at 260 nm in a Beckman DU spectrophotometer.

Results and Discussion

On a dry weight basis, the copper and nitrogen content of rice plants submitted for 30 days to increasing copper concentrations is shown in Table 1. The data show that the copper concentration in the shoots increased slowly from the 0.002 to the 1.25 mg/l Cu concentration range. Only on increasing the copper concentration to 6.25 mg/l does a 5-fold increase in shoot copper content occur. In roots, copper concentration increases slightly between the 0.002 and the 0.01 mg/l Cu-treatment, but grows linearly and with a high slope between the 0.05 and the 6.25 mg/l Cu-treatments. Nitrogen

Table 1

Copper and nitrogen concentration in rice plants.

(Each value is the mean \pm (S.E.) based on three replicates of three independent series.)

Cu treatment (mg Cu/l)	Metals concentration			
	μ g Cu/g (dw) \pm (S.E.)		mg N/g (dw) \pm (S.E.)	
	Shoots	Roots	Shoots	Roots
0.002	17.5 \pm 1.2	91 \pm 5.0	26.04 \pm 2.3	21.56 \pm 1.8
0.01	21.5 \pm 1.6	134 \pm 8.1	32.06 \pm 2.9	22.26 \pm 2.1
0.05	27.0 \pm 2.1	559 \pm 18.1	32.62 \pm 3.1	20.72 \pm 1.9
0.25	46.5 \pm 3.3	1683 \pm 34.5	35.28 \pm 3.1	24.08 \pm 2.3
1.25	95.0 \pm 6.9	2215 \pm 39.2	32.76 \pm 2.5	22.49 \pm 1.8
6.25	508.0 \pm 21.1	3380 \pm 42.5	31.09 \pm 2.2	20.54 \pm 1.7

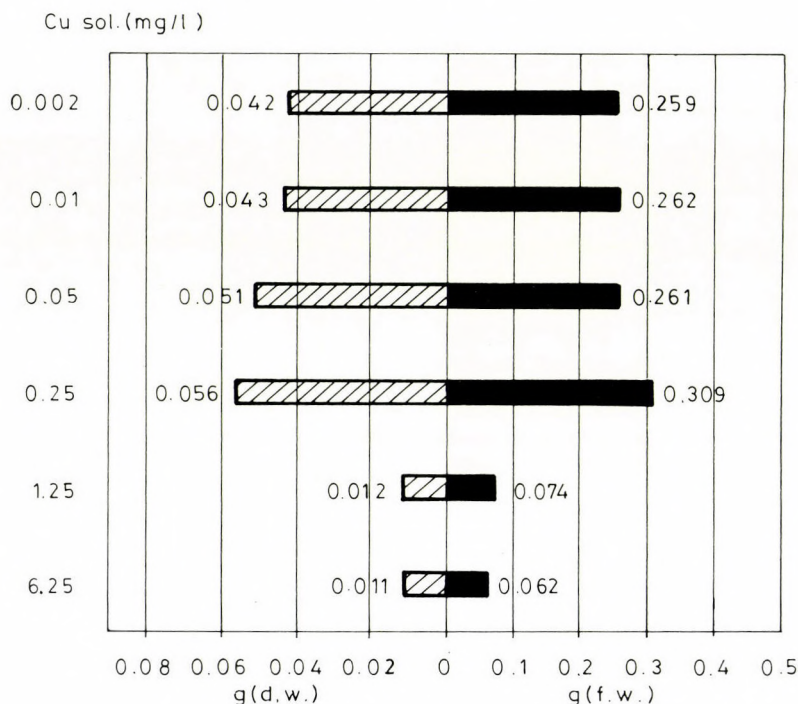


Fig. 1. Shoot biomass yields of rice plants submitted for 30 days to increasing copper concentrations. (Each value is the mean based on three replicates of three independent series for all treatments (S.E.) was equal or lower than 8%)

concentration in the shoots increased until the 0.25 mg/l Cu treatment but showed a slight decrease afterwards until the 6.25 mg/l Cu treatment. In roots, the observed changes of nitrogen concentrations did not show any correlation with nutrient copper levels.

From these data we conclude that although increasing copper levels in the nutrient solution increase copper concentration in roots and shoots, nitrogen concentration is not directly or particularly affected by those metal concentrations. It is also possible that as only on increasing the copper concentration to 6.25 mg/l does a 5-fold increase in shoot copper occur, a disruption of the mechanism(s) regulating the translocation of the metal from the root to the shoot part of the plants may occur (HENRIQUES 1990).

Shoot biomass yields of rice plants submitted for 30 days to increasing copper concentrations are shown in Fig. 1.

Table 2

Root and shoot lengths of Cu treated rice plants.
(Each value is the mean \pm (S.E.) based on three replicates
of three independent series)

Cu treatments (mg Cu/l)	Shoot lengths (cm) \pm (S.E.)	Root lengths (cm) \pm (S.E.)
0.002	43 \pm 3.0	9 \pm 0.7
0.01	43 \pm 3.0	9 \pm 0.7
0.05	39 \pm 2.5	7 \pm 0.5
0.25	32 \pm 2.5	5 \pm 0.4
1.25	23 \pm 1.5	1.5 \pm 0.1
6.25	7 \pm 0.5	2.5 \pm 0.2

One can see that the biomass yield is maximum in the 0.25 mg/l Cu treatment. However, although on a dry weight basis the biomass yield increases slightly or at least remains almost constant, from the 0.002 to the 0.25 mg/l Cu treatment, on a fresh weight basis the biomass yields are almost the same from the 0.002 to the 0.05 Cu treatment. In the last two treatments, a strong decrease in the shoot biomass is observed. From the data, one may suggest that below the 0.25 mg/l Cu treatment rice shoots tend to increase or at least maintain their dry matter production when faced with increasing copper availability.

The observation of the plants submitted to different Cu treatments reveals a progressive decrease of plant height after the 0.01 mg/l (Table 2). The shoot of the 1.25 mg/l Cu treatment also showed some chlorosis and the 6.25 mg/l Cu treatment became almost completely chlorotic. As the root systems of plants in different treatments showed reduced growth, it is possible that the cellular integrity may be affected, yet its N content is not greatly affected (Table 1).

After the 0.01 mg/l Cu treatment, the protein content of rice shoots tend to decrease progressively with increasing Cu levels, being extremely low in the 1.25 and 6.25 mg/l Cu treatments (Fig. 2). As the 0.002 mg/l Cu treatment corresponds to a deficiency situation, the 0.01 mg/l is a normal level and the 0.05, 0.25, 1.25 and 6.25 are high Cu treatments (YOSHIDA *et al.* 1976) and because Cu does not induce N deficiency on rice (Table 1), it can be suggested that copper excess induces a decrease in protein content on rice plants, an observation similar to that previously reported for other plants (BARUA and JANA 1986; FERNANDES and HENRIQUES, *in press*).

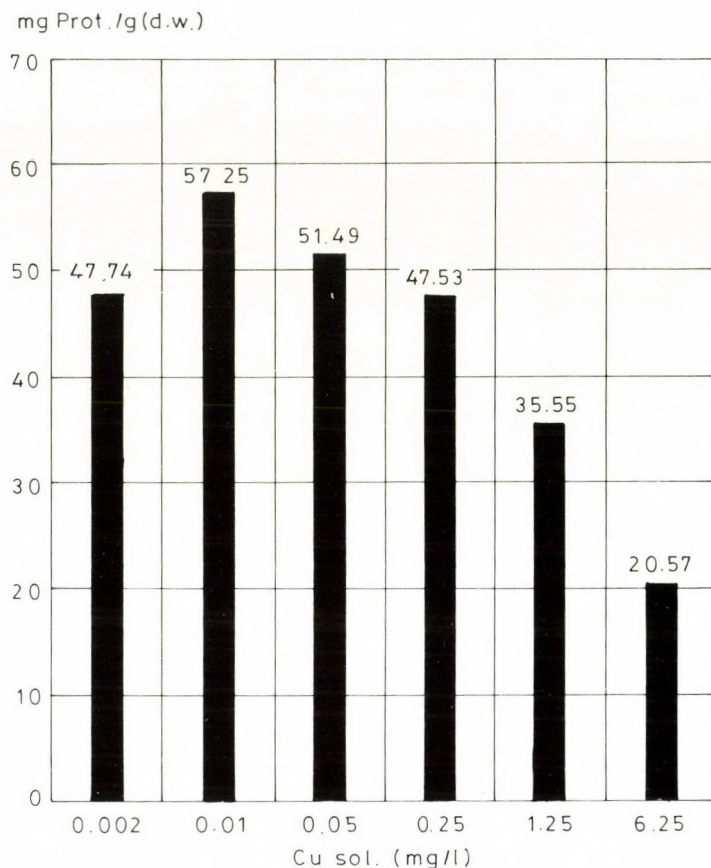


Fig. 2. Protein content of rice plants submitted for 30 days to increasing copper concentrations. (Each value is the mean based on three replicates of three independent series; for all treatments (S.E.) was equal or lower than 8%)

The electrolytic conductance of rice shoots is shown in Fig. 3. Considering that an increase in membrane permeability is associated with an increase in electrolytic conductance (KETCHIE, BEEMAN and BALLARD 1972), it can be suggested that an increase of copper concentration is associated with an increase in membrane permeability in rice shoots. When compared to the 0.01 mg/l treatment (control), the electrolytic conductance of the 1.25 and 6.25 Cu treatments (Fig. 3) shows a 2-fold increase which indicates that the deleterious effect of high Cu levels on the membrane permeability is particularly marked in rice shoots.

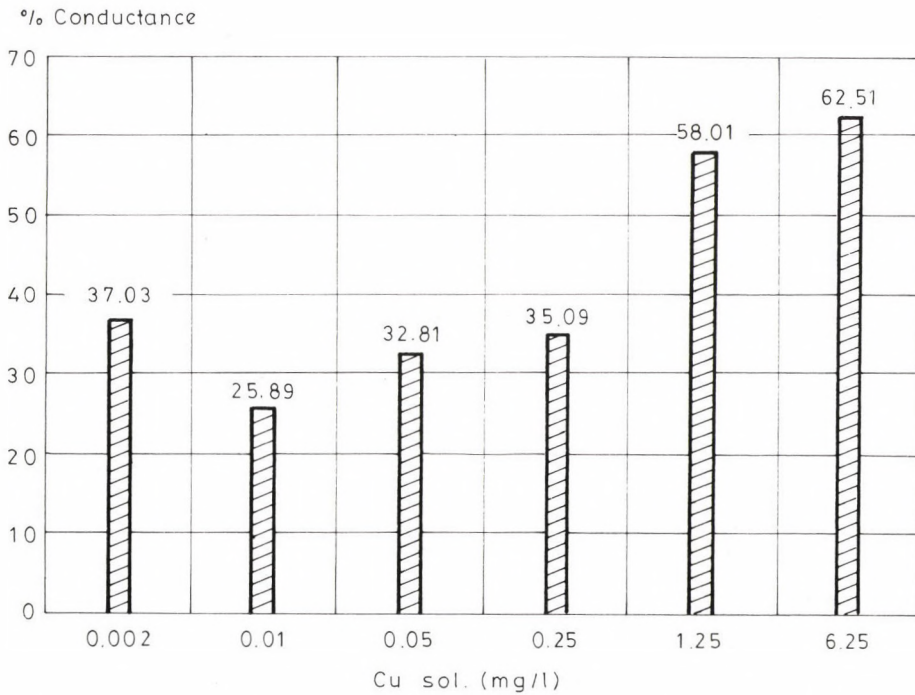


Fig. 3. Electrolytic conductance of rice shoots. (Each value is the mean based on three replicates of three independent series; for all treatments (S.E.) was equal or lower than 8%)

Protease and acid RNase activities of rice shoots are shown in Fig. 4. The activity of rice shoot protease does not respond linearly to an increasing copper content. However, when compared to the 0.01 mg/l treatment, the protease activity of the 1.25 mg/l Cu treatment shows a 2-fold increase and that of the 6.25 mg/l shows a 4-fold increase. As protease inhibitors have been identified in vegetables (NEURATH 1984), it is possible that high toxic copper levels lead to a decrease in the content of such protease inhibitors resulting in the large stimulation of protease activity observed, yet more studies are required on this subject.

Acid RNase activity tends to increase slightly from the 0.01 mg/l Cu treatment until the 0.25 mg/l treatment; however, the 1.25 and 6.25 mg/l Cu treatments, when compared with the 0.01 mg/l treatment, show a 6-fold and a 8-fold increase of that activity, respectively. The data suggest that an increase in shoot copper content is related to an increase of acid RNase activity. Considering that the enhancement of acid RNase activity is related

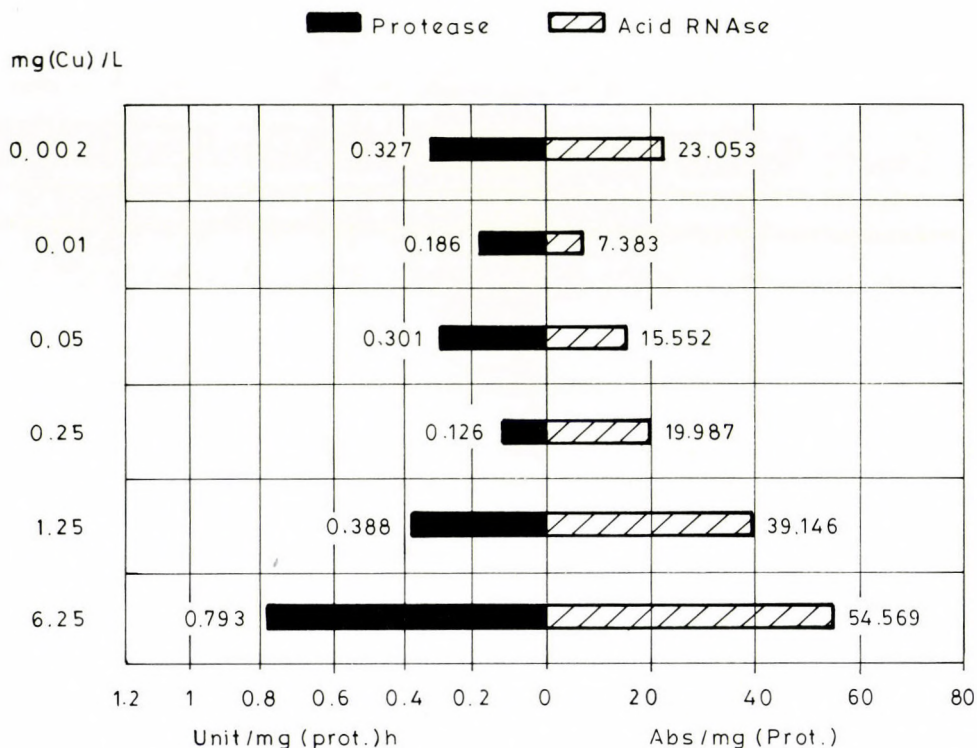


Fig. 4. Protease and acid RNase activities of rice shoots. (Each value is the mean based on three replicates of three independent series; for all treatments (S.E.) was equal or lower than 8%)

to an increase in membrane permeability (DAVE and KANNAN 1980), we suggest that an increase of copper content promotes an alteration, or even a disruption, of the rice shoot cellular membranes which, in turn, is responsible for the observed increase in acid RNase activity.

Conclusion

The relativity of the term "copper toxicity" on rice is firmly expressed from our data. Indeed, according to the biomass data (Fig. 1), growth in the 1.25 and 6.25 mg Cu/l is toxic, while according to the shoot length (Table 2) as little as 0.05 mg Cu/l is toxic. Also, while on the basis of mg protein/g (d.w.), 0.01 to 6.25 mg Cu/l caused a decrease in the concentration of tissue protein (Fig. 2), on the basis of mg protein/plant,

growth in the 0.002 to 0.25 mg Cu/l progressively increased protein yield per plant.

Although it is well established that during short-term experiments excess metals, namely copper, increase membrane permeability (BOWEN and NISSEN 1977; HASSAN and TANG VAN HUI 1976; VELTRUP 1977), such studies have not been carried in long-term experiments in which the plant is allowed to adjust to the stress situation. We show here that in such long-term experiments, as increasing copper levels do not induce nitrogen deficiency, protein content of rice shoots, tends to decrease with increasing copper levels not as a reflection of decreased N uptake and/or translocation. Indeed our data suggest that in vivo excess copper affects protein synthesis mainly by increasing membrane permeability. The observation that high levels of copper in rice shoots promote an increase in the permeability of cell membranes, suggests that in vivo this induces an enhancement of acid RNase activity. As a result, more cytoplasmic and/or membrane bound RNA is degraded, leading to a decrease in protein content of rice shoots.

Although increasing acid RNase and protease activities are general responses to plant stress observed from short-term studies, our long-term studies show that increasing copper contents in rice shoots decrease protein contents by affecting acid RNase activity. The increase of protease activity measured at the two highest copper concentrations seems to indicate that this acid hydrolase activity may not be directly related to the excess copper, but may rather constitute a response to a general change of rice metabolism under continuous metal stress.

REFERENCES

- Ambellan, E., Hollaender, V. P. (1966): A simplified assay for RNase activity in crude tissue extracts. Analytical Biochem. 17: 474-484.
- Barua, B., Jana, S. (1986): Effects of heavy metals on dark induced changes in Hill reaction activity, chlorophyll and protein contents, dry matter and tissue permeability in detached Spinacea oleracea L. leaves. Photosynthetica 20: 74-75.
- Bell, J. N. B. (1980): Response of plants to sulfur dioxide. Nature 284: 399-400.
- Bremner, J. M. (1965). In: Black, C. A., Evans, D. D., Ensminger, L. E., White, J. L., Clark, F. E. (eds): Methods of soil analysis — Part 2. Chemical and Microbiological Properties. Amer. Soc. of Agron. Inc., Publ., Madison, Wisconsin, USA, pp. 1143-1178.
- Bradford, M. M. (1976): A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein-dye binding. Analytical Biochem. 72: 248-253.

- Bowen, J. E., Nissen, P. (1977): Boron uptake by excised roots II. Characteristics and Kinetics of active uptake. Physiologia Plantarum 41: 109–115.
- Church, F. C., Porter, D. H., Catagnani, G. L., Swaisgood, H. E. (1985): An o-Phthaldehyde spectrophotometric assay for proteinases. Analytical Biochem. 146: 343–348.
- Dässler, H. G. (1979): A légszennyeződés hatása a növényzetre (Effect of air pollution on vegetation). Mezőgazd. Kiadó, Budapest.
- Dave, I. C., Kannan, S. (1980): Boron deficiency and its associated enhancement of RNase activity in bean plants. Z. Pflanzenphysiologie 97: 261–263.
- Fernandes, J. C., Henriques, F. S. (in press): Biochemical, physiological and structural effects of excess copper in plants. The Botanical Reviews.
- Govind, N. S., Mehta, B., Sharma, M., Modi, V. V. (1981): Protease and carotenogenesis in Blakeslea trispora. Phytochemistry 20: 2483–2485.
- Hassan, M. M., Tang van Hui (1976): Kinetics of zinc uptake by Citrus roots. Z. Pflanzenphysiologie 79: 177–181.
- Henriques, F. S. (1990): Ultrastructural effects of copper toxicity in rice plants. XXV Reun. Soc. Portug. Microscop. Electr., Porto, pp. 43–44.
- Ketchie, D. O. (1969): Methods of determining cold hardiness and cold injury in citrus. Proceedings First International Citrus Symposium 2: 559–563.
- Ketchie, D. O., Beeman, C. H., Ballard, A. L. (1972): Relationship of electrolytic conductance to cold injury and acclimation in fruit trees. Journ. of Amer. Soc. Hortic. Sci. 97: 403–406.
- Kovács, M. (1975): A környezetvédelem biológiai alapjai (Biological basis of environment protection). Mezőgazd. Kiadó, Budapest.
- Kovács, M. (1985): Pollution Control and Conservation. Akad. Kiadó, Budapest.
- Kowalski, D., Leary, T. R., McKee, R. E., Sealock, R. W., Wang, D., Laskowski, M. (1974). In: Fritz, H., Tschesche, H., Greene, L. J., Truscheit, E. (eds): Proteinase Inhibitors. Springer Verl., N. Y. pp. 311–324.
- Neurath, H. (1984): Evolution of proteolytic enzymes. Science 224: 350–357.
- Ohki, K. (1975): Lower and upper critical zinc levels in relation to cotton growth and development. Physiol. Plant. 35: 96–100.
- Shortman, K. (1961): Studies on cellular inhibitors of ribonuclease-inhibitor system and the purification of the inhibitor from rat liver. Biochim. et Biophysica Acta Sci. 51: 37–45.
- Veltrup, W. (1977): The uptake of copper by barley roots in the presence of zinc. Z. Pflanzenphysiologie 36: 201–206.
- Yoshida, S., Forno, D. A., Cook, J. H., Gomes, K. A. (1976): Routine procedure for growing rice plants in culture solution. In: Laboratory manual for physiological studies of rice. The Internat. Rice Res. Inst., Los Baños, Laguna, Philippines. Ed. 3. p. 61–65.

INFLUENCE OF LASER BEAM OF DIFFERENT WAVELENGTHS ON THE PROTEIN AND NUCLEIC ACID CONTENT IN GERMINATING ZEA MAYS L.

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Research into the influence of laser beam of three different wavelengths (λ = 337.1 nm, 510 nm, 632.8 nm) on germinating maize seeds was carried out investigating some parameters of metabolic processes in seedlings. As our results show, during the period of investigation (1-6 days) the laser irradiation of 632.8 and 510 nm wavelengths (performed in the 24th hour of germination) did not modify the protein content of either the embryo or the endosperm, compared with control seeds. At the same time, the light of 337.1 nm increased the soluble protein content in the embryo, depending on the degree of dose. The level of free α -NH₂-N has also changed to the highest degree after UV-A radiation. RNA and DNA contents were not modified by any of these irradiations.

Introduction

Germinating seed is considered to be a tool easy to manage in order to test the effects of irradiation and environmental elements on metabolic processes. In this heterotrophic phase of development, by the separation of seed parts, synthetic and catabolic processes can be investigated with the help of some properly chosen parameters (BEWLEY and BLACK 1984).

In our previous experiments the influence of He-Ne laser irradiation (λ = 632.8 nm) was measured first of all in the change of glucose content (KEREPESI *et al.* 1989). In further experiments we studied whether irradiations of different wavelengths modified different metabolic processes, and if the degree of deviations depended on the applied energy.

Material and Methods

Investigated material: Zea mays L. convar. saccharata. Seeds were germinated, treated, and samples were prepared as described earlier (KEREPEŠI et al. 1989).

Table 1
Treatments applied on the studied materials

Equipment	Mode of operation	λ (nm)	P	Repetition frequency	J/seed	Radiation time (s)
MOM-77	permanent	632.8	5.6 mW	—	0.1	18
					1.0	180
NDL-2 JPTE	impulse	510	0.232 mJ/imp.	10 Hz	0.5	210
NL-300 JPTE	impulse	337.1	2.5 mJ/imp.	10 Hz	0.1 1.0	4 40

Results and Discussion

The study of metabolic processes in germinating seed is an experimental system easy to be reconstructed and in which the laser effect can be characterized quantitatively as well. Changes are fast, there is no photosynthesis yet, and independent measurements permit the statistical analysis of results. By the separation of each seed to endosperm and embryo the influence can be observed better; that is why we examined the change of metabolic processes in these two parts of seed with different functions separately.

Table 2

The free α -NH₂-N content in the embryo (a) and endosperm (b) of germinating Zea mays in the percent of control

λ day	337.1 nm				510 nm		632.8 nm			
	0.1 J		1.0 J		0.5 J		0.1 J		1.0 J	
	a	b	a	b	a	b	a	b	a	b
1	—	—	—	—	—	—	96	100	98	93
2	163	—	145	—	92	103	98	109	92	80
3	134	—	69	—	110	96	111	110	67	91
4	124	95	115	203	89	84	93	118	67	81
5	133	158	116	287	114	143	—	—	—	—

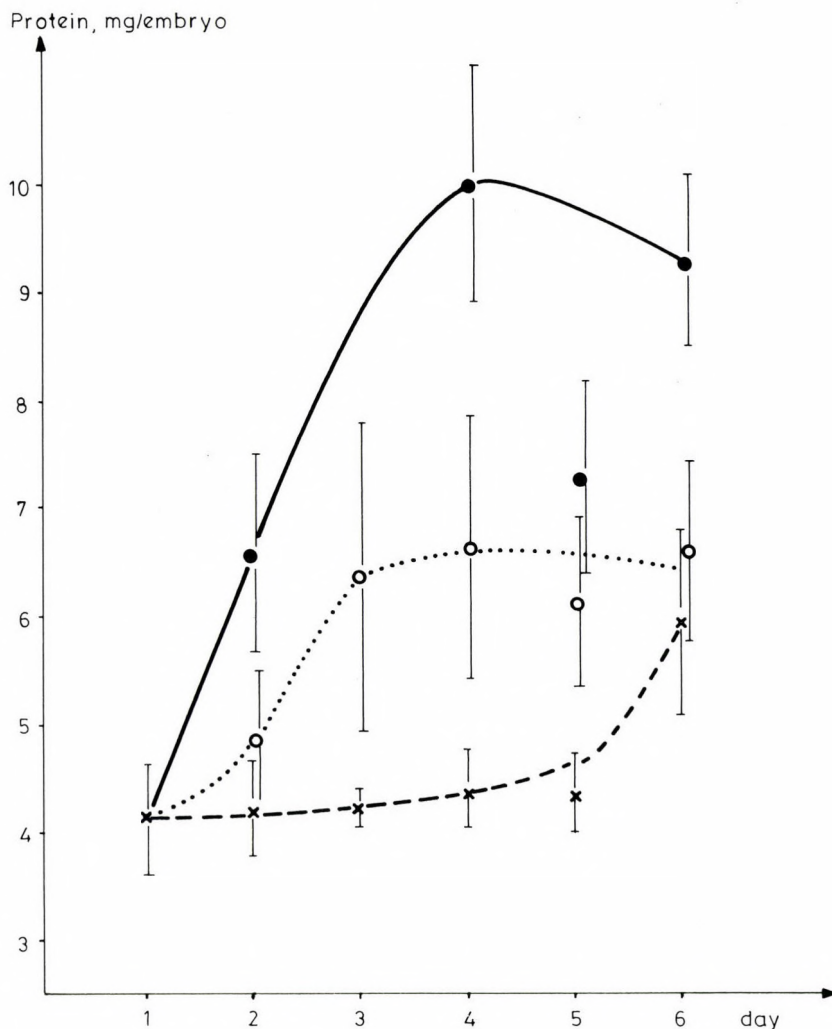


Fig. 1. The influence of UV (337.1 nm) laser light on the soluble protein content of germinating *Zea mays* embryo. (x) control; (o) 0.1 J; (●) 1.0 J

During the period of the investigated 1-6 days RNA and DNA contents have changed according to the growth curve in each examined group (KEREPESI 1989; INGLE *et al.* 1965). Applied irradiations have failed to have any influence.

We studied the change of free amino acid content of the seeds by measuring the total α -NH₂-N content. Table 2 shows these results in the percents of the control measurements.

After the irradiation of 632.8 nm we did not measure significant differences in the endosperm, while in the embryo the 1 J irradiation caused a lower α -NH₂-N level, compared with control seeds. On the 3rd and 4th days of germination this change was more than 30%. The effect of laser beam of 510 nm was not significant in either seed parts with regard to standard deviation. The irradiation of 337.1 nm laser beam increased the free α -NH₂-N content in both seed parts, mainly in the endosperm. The influence depends on the dose; applying 1 J we measured higher values in contrast to 0.1 J irradiation.

The irradiation of 632.8 nm laserbeam did not modify the protein content of either the embryo or the endosperm. The laser beam of 510 nm caused measurable difference in the endosperm only, in which we measured higher protein content on the 2nd and 3rd days of germination, compared with control seeds. At the same time, the irradiation of 337.1 nm increased the quantity of soluble proteins in the embryo, depending on the dose (Fig. 1).

Summary

Comparing the biochemical effects of the applied irradiation it can be said that laser-beam of all the three wavelengths has caused changes of different degrees in germinating seeds during the investigated 1-6 days.

The quantity of nucleic acids (RNA, DNA) was not influenced by irradiation. The most significant differences were measured in the mobilization of stored nutrients. The greatest differences were measured after UV irradiation, compared with control values which caused the increase of protein utilization (soluble protein content, free α -NH₂-N content) (Fig. 1). As a result of He-Ne laser beam the examined values of carbohydrate metabolic processes have decreased (KEREPESEI *et al.* 1989). The α -NH₂-N level of the embryo has decreased, in contrast to UV irradiation (Table 2). After the irradiation of 510 nm we did not measure significant changes.

Evaluating the investigations it must be noted that the laser beams, applied for irradiation, differed in several parameters. For example, in case of similar energy doses the irradiating light's power, and this way the duration of irradiation were different. With regard to the influence of irradiation it can also be important whether the sample gets the actual dose in a series of high power, short impulses with low repetition frequency (impulse mode of operation), or it is the result of low power permanent irradiation (permanent mode of operation).

REFERENCES

- Bewley, J. D., Black, M. (1984): Seeds: physiology of development and germination. Plenum Press, N. Y., London.
- Kerepesi, I., Tóth, M., Sánta, I., Kozma, L., Klujber, L. (1989): Influence of He-Ne laser irradiation on Zea mays L. seeds. Working Papers (in press).
- Kerepesi, I. (1989): A lézertény hatása a csírázó kukoricamag anyagcsere-élettani folyamataira. Egyetemi doktori értekezés. (Effect of laser-radiation on metabolism processes of germinating maize seeds. Univ. doctor thesis.)
- Ingle, J., Hageman, R. H. (1965): Metabolic changes associated with the germination of corn. II. Nucleic acid metabolism. Plant Physiol. 40: 48-53.

DRY MATTER ACCUMULATION AND DISTRIBUTION, SOIL AND ^{15}N LABELLED FERTILIZER NITROGEN UPTAKE AND REDISTRIBUTION IN MAIZE

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A two-year pot experiment was carried out in a growth-house to investigate the dry matter (DM) accumulation, distribution and redistribution in maize (*Zea mays* L.). The uptake, distribution and translocation of soil and fertilizer nitrogen were also studied. The total dry matter weight (DMW) of aerial plant parts increased to 108 days after emergence (DAE) and then with the exception of grain, decreased to the final harvest. In the average of two years most DM was transported from the stalk (69.41%) and least from the leaf-sheet (1.69%) to the grain. In both years up to the grain formation developmental stage the nitrogen concentration was highest in the leaf-blades then the highest nitrogen concentration was overtaken by grain. More than half (58.91%) of the total nitrogen of grain was translocated from the aerial plant parts in the average of the two experimental years. The percentage of fertilizer and total nitrogen distribution in aerial plant parts was close at the final harvest in both years. Calculating for the whole plant, including the roots, the two-year mean value of the fertilizer nitrogen utilization was 64.85%.

Introduction

Dry matter (DM) accumulation of the whole maize plant proceeds up to the developmental stage of biological maturity. This DM accumulation time is shorter at different plants parts since after a certain developmental stage a significant part of carbohydrate stored in various plant parts is translocated to the developing grain.

This carbohydrate is translocated in the maize plant as sucrose (LOOMIS 1945).

The most important nutrient element, the nitrogen is also accumulated in different plant parts, but its accumulation rate and redistribution precedes that of DM (DEBRECZENI and SZLOVÁK 1984). Since the nitrogen accumulation and redistribution may largely affect the grain yield and its quality a number of experiments were carried out to study the nitrogen uptake, distribution, redistribution and utilization (GYŐRI 1987; HANWAY 1962;

HAY et al. 1953; MENGEL and BARBER 1974; NAGY 1986; POLLMER et al. 1979; SAYRE 1948; SZLOVÁK 1974; SZLOVÁK and DEBRECZENI 1988).

The objective of this experiment was to examine the dynamics of DM accumulation, distribution and redistribution, the nitrogen uptake from both soil and fertilizer and also its utilization.

Material and Method

In both years (1984 and 1985) the maize seeds were planted on 7 May. In the 20 x 25 cm white enamel painted modified Mitscherlich culture pots for 6 kg absolute dry soil, air dry alluvial-meadow surface soil (Szarvas, Bikazug) was placed. The maximum waterholding capacity of the soil was determined in laboratory and a value of 49.7% was obtained (expressed in weight % of absolute dry soil). Other main characteristics of the soil used in the experiment: pH (H_2O): 5.95, pH (KCl): 5.65, total salt: 0.07%, humus %: 2.17, total N: 0.21.

P_2O_5	AL-P		83.3 ppm
K_2O	Al-K	method	216.2 ppm
K_A	soil plasticity index according to Arany		46.4

The soil on which the hybrid MSC 3780 Pioneer maize plants developed was filled with water to 70% of its maximum water-holding capacity at daily waterings.

The active ingredients of fertilizers per pot were as follows: N: 2.4 g (ammonium-nitrate), P_2O_5 : 1.2 g (superphosphate), K_2O : 1.2 g (potassium chloride).

NH_4NO_3 , containing 10 atom % ^{15}N , was used as nitrogen fertilizer. Both cation and anion contained ^{15}N .

Five seeds were sown per pot. After emergence the plants were thinned to one in each pot. There were 7 replicates in 1984 and 10 in 1985.

The plants were harvested 17 times in 1984 and 12 times in 1985. In 1984 stalk and tassel was weighed and analyzed together in 1985 separately. In both years shank is included in husks. At harvests the roots were washed out of the pot soil. After separation, the plant parts were dried at an oven temperature of 60 °C. The drying continued until there was no more change in the subsequent weight measurements. For the total nitrogen determination the Kjeldahl method and for the analysis of isotope ratios a Straton Isonitromat 5201 automatic nitrogen analyser (GDR) was used.

Results and Discussion

Dry matter accumulation and translocation

Figure 1 shows the DM accumulation and its distribution among plant parts during the growth of maize in 1984. The total dry matter weight (DMW) of plant parts increased to 28 July, that is to 108 days after emergence (DAE) and then with the exception of grain decreased to the final harvest, 129 DAE. At the peak the DMW of stalk was 25.15% of the total aerial parts of the plant (Table 1). Lowest value was obtained at husks (6.40%). Already at this developmental stage the DMW of grain was the highest (37.88%). The DMW of roots from now on also decreased. Most DM was transported from the

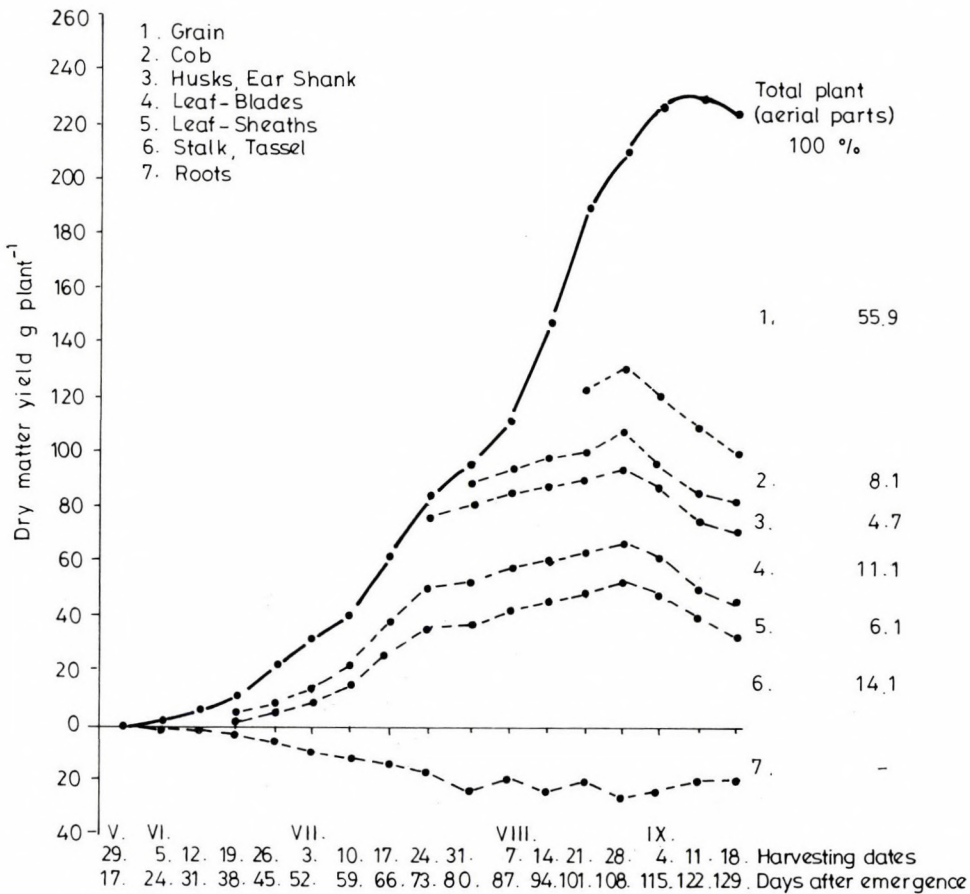


Fig. 1. Dry matter accumulation of maize (1984)

Table 1

Dry matter distribution (per cent) in plant parts with the onset of N and DM depletion in the shoot

Year	Days after emergence	Plant parts					
		Stalk	Leaf-sheaths	Leaf-blades	Tassel	Husks	Cob
1984	73	43.60	17.44	34.78		4.18 ^x	
	108	25.15	6.81	12.75		6.40	11.00
1985	64	46.40	20.00	33.70			
	107	20.89	6.18	12.32	0.90	6.22	11.21

^xSum of Husks, Cob and Grain

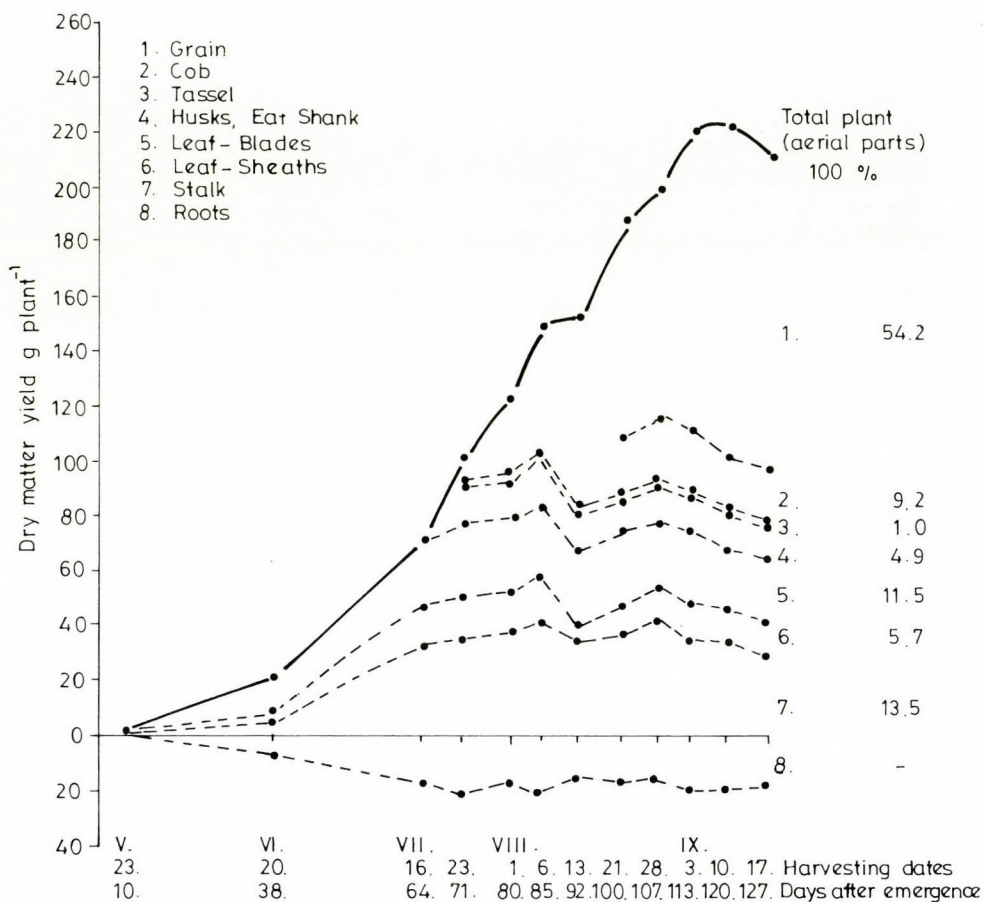


Fig. 2. Dry matter accumulation of maize (1985)

stalk to the grain (67.55%, Table 2) and least from the leaf-sheaths (2.03%). The root DM decrease (5.43 g) was not taken into account in these calculations. From the harvest 108 DAE the total DM increase of aerial parts was only 15.16 g while the grain DM increase reached 46.13 g. This could happen only if 30.97 g of DM was translocated to the grain. Among the plant parts only the grain DMW increased to the final harvest. The final DM distribution per cent among plants parts is presented in Fig. 1.

In 1985 the DM distribution among plant parts was similar to that observed in 1984 (Fig. 2). Like in 1984 about at the same time (107 DAE) the total DMW of aerial plant parts with the exception of grain steadily declined to the final harvest.

Table 2

Dry matter translocation (per cent) from plant parts to the grain from the time of maximum DMW (28 August) of aerial part of plant

Year	Plant parts				
	Stalk	Leaf-sheaths	Leaf-blades	Husks	Cob
1984	67.55	2.03	5.65	8.94	15.85
1985	71.27	1.35	0.70	11.43	15.28
Average	69.41	1.69	3.18	10.19	15.57

At the start of the steady weight decrease (107 DAE) to the end of the growth the DMW of stalk made up 20.89% of the DMW of all aerial plant parts (Table 1). The DMW of other plant parts was close to those registered in 1984. Also the DMW percentages of plant parts at the final harvest were alike in both years (Figs 1 and 2). If these percentages are compared to the data obtained in an earlier experiment (SZLOVÁK 1983) only relatively small differences occur.

With the onset of DMW decline of the aerial parts, with the exception of grain, like in 1984, there was only a slight total DM increase in 1985 (18.12 g). As in 1984 most DM (71.27%) was transported from stalk to the grain (Table 2). The DMW increase of grain (30.67 g) was significantly lower in 1985, only 66.49% of that in 1984. The grain increased with 36.77% of its final weight in 1984 and only with 26.64% in 1985.

In 1985 very likely there was or were certain factors which limited the translocation of DM from plant parts to grain. This limitation manifested itself also in the lower final grain yield.

Nitrogen accumulation and translocation

At 73 DAE in 1984 the stalk contained 0.48 g, leaf-sheets 0.21 g and leaf-blades 0.71 g nitrogen (Fig. 4). Its percentage distribution in the aerial parts of plant taking into account the 0.06 g nitrogen found in ear and husks is presented in Table 3. After 73 DAE the total nitrogen content of stalk and leaves decreases. Maximum nitrogen content in roots was observed one week later than in stalk and leaves. The rapid loss of nitrogen from the stalk, leaf-sheets and leaf-blades is concurrent with the onset of rapid grain development. This translocation of total nitrogen proceeds to the final harvest.

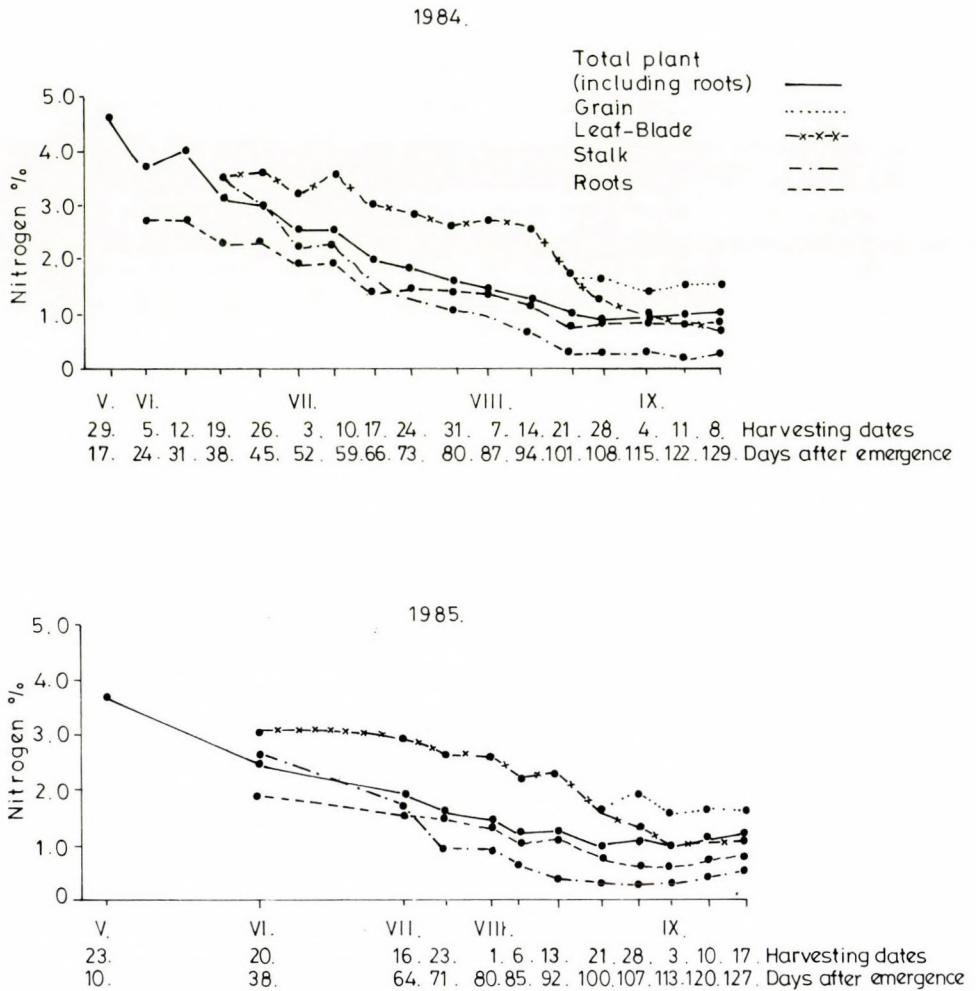


Fig. 3. Nitrogen concentration changes in maize during the growth season (1984 and 1985)

At the start of nitrogen redistribution that is 73 DAE the aerial parts of plant absorbed already 60.33% of their final total nitrogen. More than half (59.41%) of the grain nitrogen was translocated from aerial plant parts. The percentage contribution of plant parts to the translocated nitrogen was as follows: Stalk: 31.67%, Leaf-sheaths: 13.89%, Leaf-blades: 43.86%, Husks: 7.03% and Cob: 3.56%.

As in 1984 the peak of total nitrogen content of aerial plant parts precedes the DM accumulation in 1985 too (Fig. 5). This peak appeared 64 DAE,

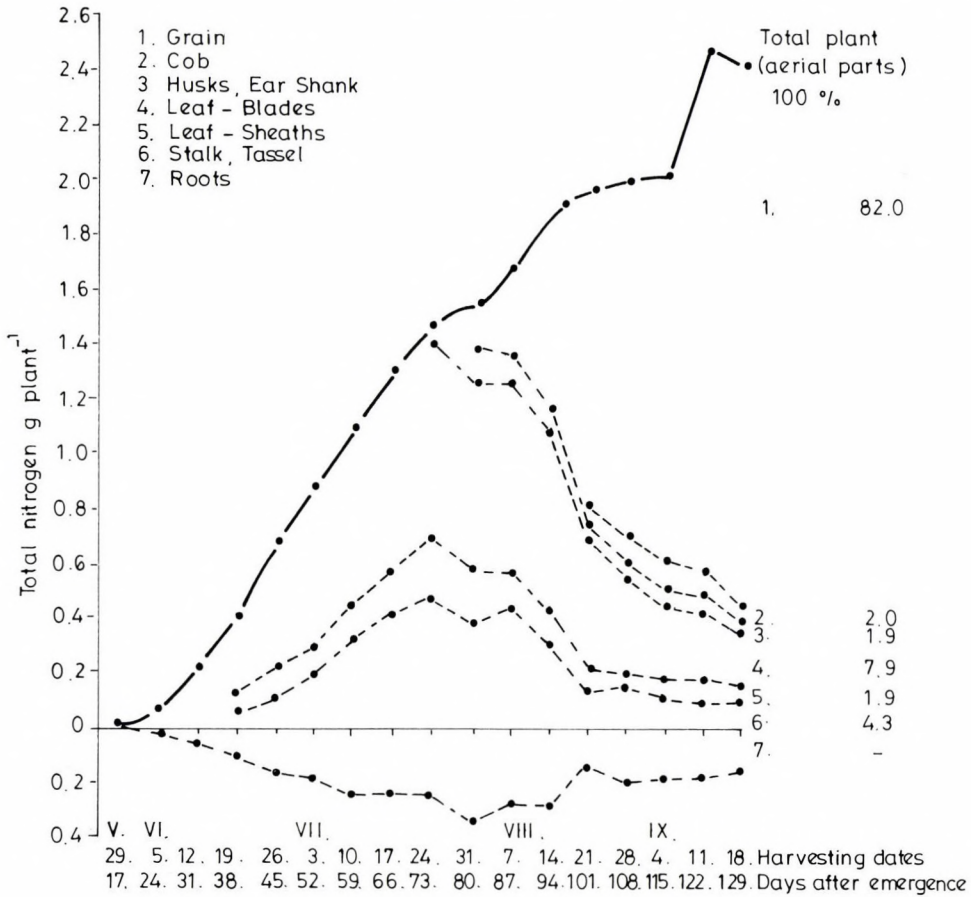


Fig. 4. Total nitrogen uptake — and translocation in maize (1984)

that is 9 days earlier than in 1984. At this stage the aerial parts absorbed 56.25% of their final nitrogen. Like in 1984 the roots contained most nitrogen one week after the maximum of aerial parts. Highest nitrogen content of leaf-blades was 0.71 g, stalk contained 0.56 g and leaf-sheaths 0.15 g. One week later the nitrogen content of roots was 0.33 g. Following the peak values much of the nitrogen was translocated (58.41% of the final nitrogen content of grain).

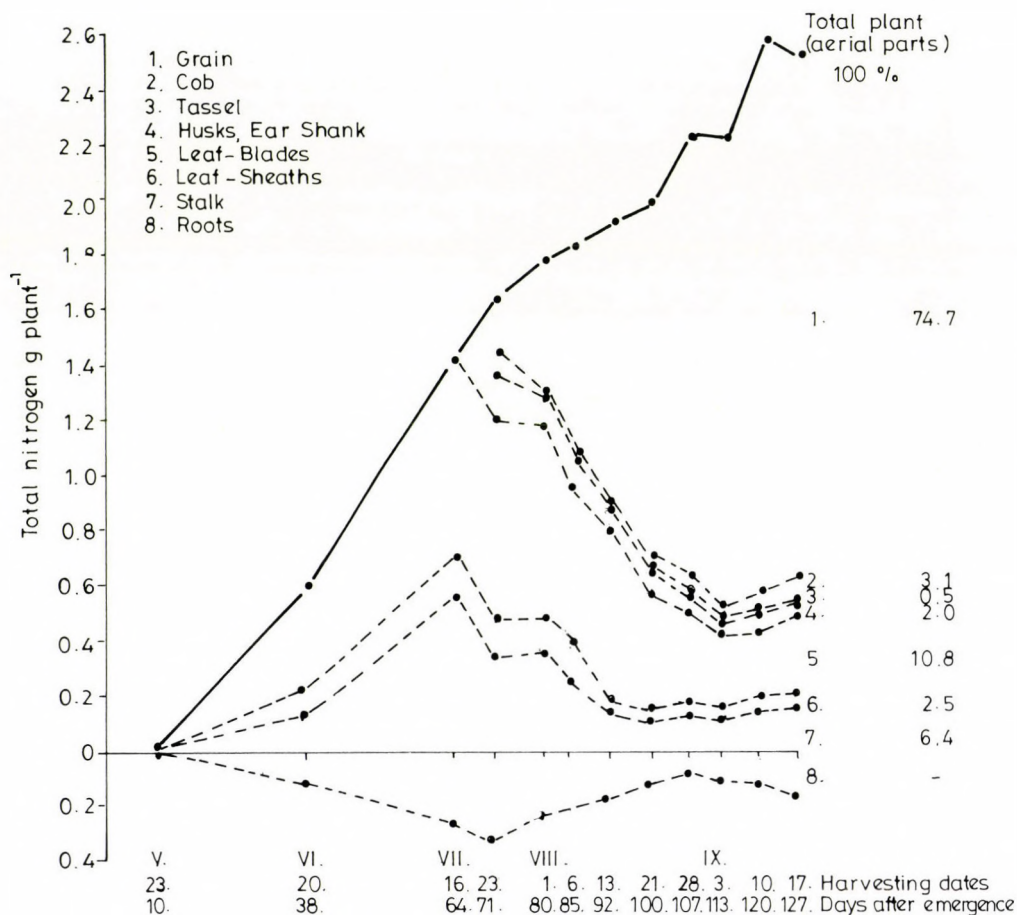


Fig. 5. Total nitrogen uptake — and translocation in maize (1985)

Nitrogen concentration

The concentration of total nitrogen (soil and fertilizer N) in different plant parts is presented in Fig. 3. As can be seen this concentration declines in both years to about 21 August (100 DAE) in all plant parts and there after only slight changes take place to the end of the growing season. In both years up to the grain formation the nitrogen concentration is highest in the leaf-blades then the highest concentration is overtaken by grain. In the first half of the growth season the roots contain the least nitrogen but in the second half with the appearance of tassel the nitrogen

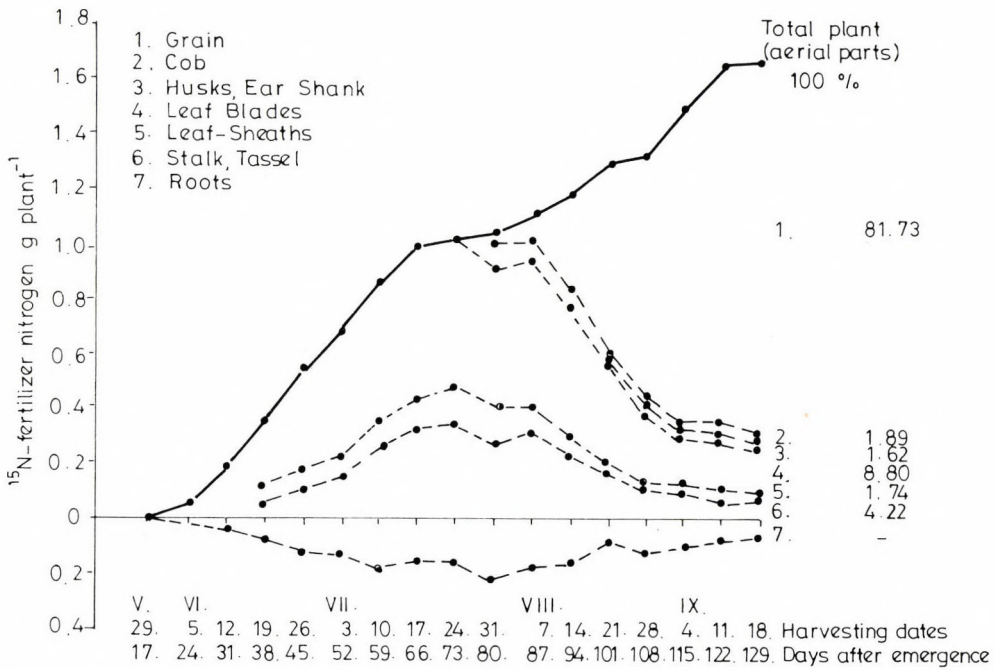


Fig. 6. Fertilizer — ^{15}N -uptake by maize (1984)

concentration of stalk decreases under that of the roots and this lower value remains until the last harvest.

Total nitrogen uptake and distribution

HANWAY (1963) pointed out that about 28 DAE the nitrogen uptake becomes rapid and continues at this rapid rate near to maturity. In our experiment the rapid nitrogen uptake started 24 DAE (Fig. 4).

By comparing the DMW and total nitrogen content of stalk, leaf-sheaths and leaf-blades (Figs 1 and 4) it is clear that the nitrogen peak precedes that of DM accumulation maximum by 35 days in 1984 and by 43 days in 1985.

Fertilizer nitrogen uptake and distribution

The use of ^{15}N -labelled fertilizer made possible to separate the nitrogen originating from fertilizer and soil. The curves presenting the dynamics of fertilizer ^{15}N uptake are running similarly to those of total nitrogen uptake in 1984 (Figs 4 and 6). The peaks appear at the same date

Table 3

Total nitrogen distribution (per cent) in plant parts with the onset of N and DM depletion in the shoot

Year	Days after emergence	Plant parts					
		Stalk	Leaf-sheaths	Leaf-blades	Husks	Cob	Grain
1984	73	32.88	14.38	48.63		4.11 ^X	
	108	7.91	1.99	17.27	2.64	5.27	64.91
1985	64	39.42	10.51	50.07			
	107	5.40	2.22	14.88	2.09	3.58	71.68

^XSum of Husks, Cob and Grain

Table 4

Fertilizer nitrogen distribution (per cent) in plant parts with the onset of N and DM depletion in the shoot

Year	Days after emergence	Plant parts					
		Stalk	Leaf-sheaths	Leaf-blades	Husks	Cob	Grain
1984	73	33.18	14.22	52.60			
	108	7.68	2.60	18.69	2.72	1.96	66.35
1985	64	39.00	9.99	51.00			
	107	5.29	2.17	15.21	2.01	3.28	71.85

for the aerial parts as well as for the roots. Also the percentage distribution of fertilizer nitrogen in plant parts at 73 and 108 DAE was similar to that measured for the total nitrogen (Tables 3 and 4). The only major difference was observed at cob 108 DAE. But even this difference almost disappeared at the final harvest.

In 1985 also the fertilizer nitrogen content of the various plant parts is similar to the total nitrogen uptake at 64 and 107 DAE (Tables 3 and 4). The curves presenting the dynamics of fertilizer nitrogen uptake are running similarly to those of total nitrogen uptake (Figs 5 and 7) like in 1984. The percentage of fertilizer and total nitrogen distribution in aerial plant parts, as in previous year is close at the final harvest.

¹⁵N-fertilizer utilization

The dynamics of ¹⁵N-fertilizer utilization by plant parts is presented in Figs 8 and 9. In 1984 the best nitrogen utilization by leaf blades was

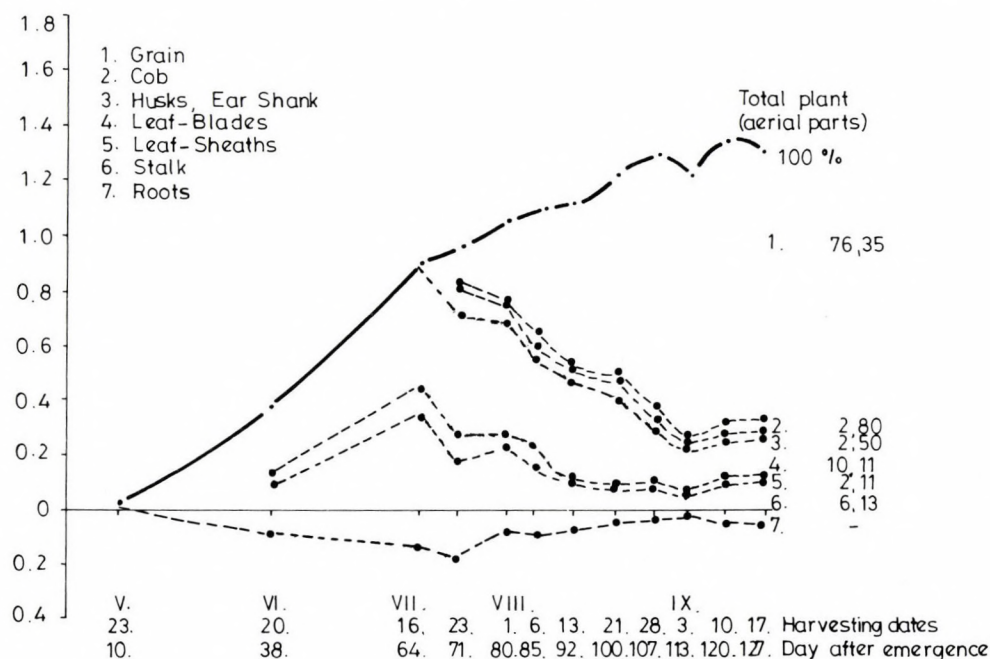


Fig. 7. Fertilizer — ^{15}N -uptake by maize (1985)

registered at the harvest of 66 DAE (Fig. 8). For stalk the highest value was observed 73 DAE, that is at the peak of nitrogen accumulation in stalk, leaf-sheaths and leaf-blades. In 1985 leaf-blades utilized the nitrogen best 71 DAE and the stalk 64 DAE (Fig. 8). In both years the nitrogen accumulation peak coincided with the best nitrogen utilization by stalk. While the nitrogen utilization by stalk was practically the same in 1984 as well as in 1985, the leaf-blades utilized better the nitrogen in 1984. Nitrogen utilization by leaf-blades was higher than by stalk in both years.

In the second experimental year the fertilizer nitrogen uptake was only 81.18% of that in the first year. If only the aerial parts were taken into account this percentage was slightly lower (80.77%). The lower fertilizer uptake in 1985 was well expressed in the fertilizer utilization. While in 1984 the nitrogen fertilizer utilization was 71.6% including the root nitrogen content in the calculations and slightly less (68.5%) when only the aerial parts were taken into account, in 1985 these values were definitely lower, 58.1 and 55.3% correspondingly. This percentage is similar to the percentage reported by MEISINGER *et al.* (1985). Using the difference

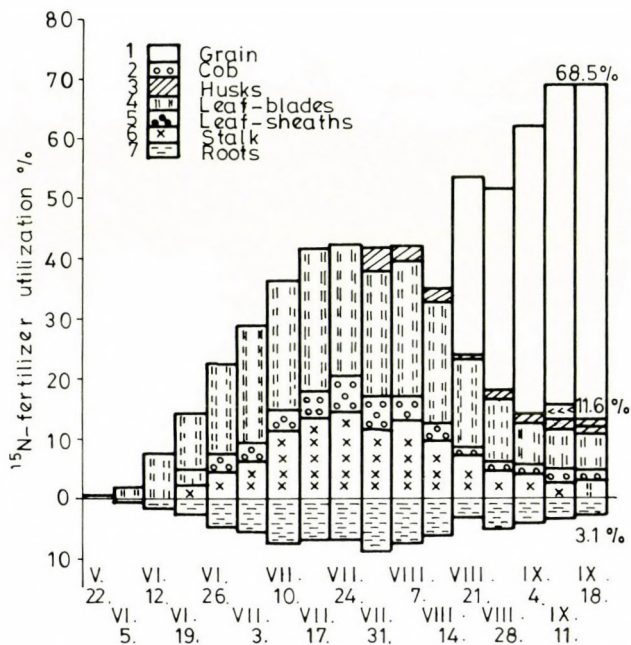


Fig. 8. ^{15}N -fertilizer utilization of maize (1984)

method for nitrogen utilization calculations and also a different maize variety at optimum water and nutrient supply SZLOVÁK (1986) obtained higher nitrogen utilization values than observed in the present study. RAO and SHINDE (1984) in their experiment using the "difference" method also found a higher nitrogen recovery than obtained by ^{15}N -labelled isotope method. This overestimated recovery is mainly due as different studies (WESTERMAN and KURTZ 1973; PATRICK and REDDY 1976; REDDY and PATRICK 1976) demonstrated to the increased uptake of native soil nitrogen by plants receiving fertilizer nitrogen compared to the plants receiving no fertilizer nitrogen.

Better nitrogen utilization in 1984 than in 1985 also coincided with a higher (108.95%) grain yield. The lower grain yield in 1985 showed a slightly higher total nitrogen concentration in grain. But even so the total nitrogen content of grain in 1984 was 105.58% compared to that of 1985.

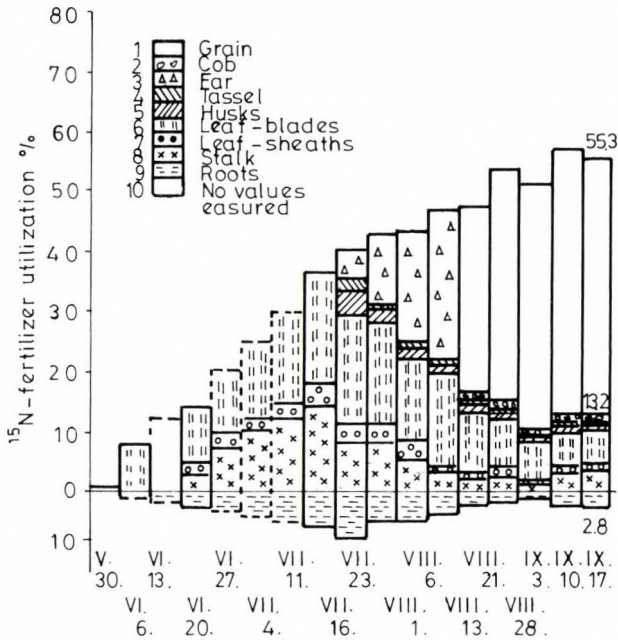


Fig. 9. ^{15}N -fertilizer utilization of maize (1985)

REFERENCES

- Debreczeni, K., Szlovák, S. (1984): Studies on the N uptake of maize (*Zea mays* L.) using ^{15}N labelled nitrogen fertilizer. 9th World Fertilizer Congress Proceedings, Budapest, 2: 167-169.
- Györi, Z. (1987): Az évjárat, a műtrágyázás és az öntözés hatása szántóföldi növényeink tápanyagtartalmára és minőségére (The effect of weather, fertilization and irrigation upon nutrient content and quality of field crops). Thesis Univ. of Agricult. Sci., Debrecen.
- Hanway, J. J. (1962): Corn growth and composition in relation to soil fertility. II. Uptake of N, P and K and their distribution in different plant parts during the growing season. Agron. J. 54: 217-222.
- Hanway, J. J. (1963): Growth stages of corn. Agron. J. 55: 487-492.
- Hay, R. E., Early, E. B., DeTurk, E. E. (1953): Concentration and translocation of nitrogen compounds in the corn plant (*Zea mays* L.) during grain development. Plant Physiol. 28: 606-621.
- Loomis, E. E. (1945): Translocation of carbohydrates in maize. Science 101: 398-400.
- Meisinger, J. J., Bandel, V. A., Standord, G., Legg, J. O. (1985): Nitrogen utilization of corn under minimal tillage and moldboard plow tillage. I. Four-year results using labelled N fertilizer on an Atlantic Coastal Plain Soil. Agron. J. 77: 602-611.

- Mengel, D. B., Barber, S. A. (1974): Rate of nutrient uptake per unit of corn root under field conditions. Agron. J. **66**: 399–402.
- Nagy, J. (1986): A tápanyag- és vízellátás hatása a kukoricahibridek termésére (The effect of nutrient and water supply upon the yield of maize hybrids). Thesis Univ. of Agricult. Sci. Debrecen.
- Patrick, W. H. (Jr.), Reddy, K. R. (1976): Fate of fertilizer nitrogen in a flooded rice soil. Soil Sci. Soc. Am. J. **40**: 678–681.
- Pollmer, W. G., Eberhad, D., Klein, D., Dhillon, B. S. (1979): Genetic control of nitrogen uptake and translocation in maize. Crop Sci. **19**: 82–86.
- Reddy, K. R., Patrick, W. H. (Jr.) (1976): Yield and nitrogen utilization as affected by method and time of application of labelled nitrogen. Agron. J. **68**: 965–969.
- Rao, K. A., Shinde, J. E. (1984): Turnover of ^{15}N -labelled urea in various rotations of groundnut, sorghum and pigeon pea crops. Plant and Soil **79**: 159–167.
- Sayre, J. D. (1948): Mineral accumulation in corn. Plant Physiol. **23**: 267–281.
- Szlovák, S. (1974): The use of weighing lysimeters in the study of evapotranspiration and nutrient uptake of maize. Factory podmienjuje efektivnost závlah II. Agromicko-biologicke problemy závlah. Bratislava, pp. 29–36.
- Szlovák, S. (1983): The effect of increasing nitrogen doses upon dry matter production, transpiration and water utilization of maize plants. Acta Bot. Hung. **29**: 293–306.
- Szlovák, S. (1986): The effect of nitrogen doses and different water supply upon nitrogen utilization of maize. Acta Agron. Hung. **35**: 239–255.
- Szlovák, S., Debreczeni, K. (1988): Kukoricahibridek szárazanyagtömegének, transzspirációjának és nitrogén felvételének vizsgálata (Investigation of dry matter accumulation, transpiration and nitrogen uptake in maize hybrids). Manuscript. Szarvas. Research Inst. for Irrigation.
- Westerman, R. L., Kurtz, L. T. (1973): Priming effect of ^{15}N -labelled fertilizers on soil nitrogen in field experiments. Soil. Sci. Soc. Am. Proc. **37**: 725–727.

INTERRELATIONSHIP OF *ORYZA GLABERRIMA* STEUD. WITH ANNUAL WILD RICES
OF THE *O. GLABERRIMA* AND *O. SATIVA* COMPLEXES:
ANALYSES OF VARIATION IN REPRODUCTIVE MORPHOLOGY

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The cultivated African *Oryza glaberrima* Steud., its wild progenitor, annual *O. barthii* Chev. and wild annual *O. stapfii* form a species complex. The wild species occur as weeds of cultivated rice. 20 accessions of wild and cultivated species were grown under standardised conditions. At the end of the vegetative phase, 26 characters of the reproductive phase were analysed by principal component analyses. Accessions were clustered into a dendrogram from a Euclidean distance matrix. The characters succeeded in separating the wild annuals from the cultivated types.

Introduction

Cultivated *O. glaberrima* Steud. originated from *O. barthii* Chev. with other wild annuals variously described and sometimes taxonomically grouped as *O. stapfii* Rosch. *O. barthii* occurs as weeds of cultivated rice fields and farmers cannot distinguish wild from cultivated forms. This means that the weeds compete with the cultivated types and both are subjected to similar selection pressures.

Cultivated plants have very large gene pools. They do not have a natural population structure and occupy artificial areas often greater and more varied than their wild progenitors (HUTCHINSON 1965). Complex selective forces have been in operation during the long period of cultivation (HAWKES 1970). There is introgressive hybridisation across breeding barriers which enlarges the gene pool even further. This very large amount of complex and constantly changing variation has to be identified, described and classified.

O. sativa L. is the Asiatic cultivated rice. CHANG (1976) proposed that it originated independently from the perennial wild progenitor *O. rufipogon* Griff. both forming a species complex with *O. nivara* Shastri et Sharma. NAVAR (1973) suggested that *O. glaberrima* evolved from *O. sativa* which was introduced into Africa around the 10th century A.D. Nomenclature

Table 1

Nomenclature of wild species closely related to O. sativa and O. glaberrima

African FormsO. barthii Chev.Syn. O. breviligulata Chev. et RoehrO. stapfii Rosch.O. longistaminata Chev.Syn. O. barthii non Chev.O. perennis Moench subsp. barthiiAsiatic FormsO. rufipogon Griff.Syn. O. perennis MoenchO. nivara Sharma et ShastrySyn. O. sativa L. var. fatua prainO. sativa f. spontanea Rosch.O. fatua Koenig nom. nud.

of the wild species closely related to the two cultivated species is given in Table 1.

Using vegetative characters alone, it has been shown that it can be difficult to distinguish between the wild and the cultivated African rice (DANIA OGBE and WILLIAMS 1978). The present study was carried out to evaluate reproductive characters in identifying and typifying the species.

Material and Methods

Accessions of O. glaberrima and annual wild rices were grown under standardised conditions in a greenhouse. At the end of the annual vegetative growth phase, 10 spikelets from plants in the flowering stage (Table 2) were observed.

Length of anthers, styles and stigmas; number of stigmas, width and colour of stigmas were recorded. When the panicles were ripe for harvesting of the seed, 16 other characters were scored — length, breadth, and width of spikelet; lemma length; thickness and length of awn; hairiness of awn and lemma; length, density, distribution pattern and colour of spikelet hairs; colour of spikelet and grain; presence or absence of shattering of the spikelet and the presence or absence of an appendage between the apiculi.

Table 2

	Accession List	*Species	Source
1	GLB 100144	<u>O. glaberrima</u>	IRRI, Phillipines
2	GLB 101921	<u>O. glaberrima</u>	IRRI, Phillipines
3	GLB 101882	<u>O. glaberrima</u>	IRRI, Phillipines
4	GLB 101895	<u>O. glaberrima</u>	IRRI, Phillipines
5	BTH 100929	<u>O. barthii</u>	IRRI, Phillipines
6	STF P7237987	<u>O. stapfii</u>	US Dept. Agric.
7	GLB 1	<u>O. glaberrima</u>	Sierra Leone
8	STF 101050a	<u>O. stapfii</u>	US Dept. Agric.
9	GLB 101922	<u>O. glaberrima</u>	IRRI, Phillipines
10	BTH 101252	<u>O. barthii</u>	IRRI, Phillipines
11	SPT 100847	<u>O. spontanea</u>	IRRI, Phillipines
12	SPT 100943	<u>O. spontanea</u>	IRRI, Phillipines
16	GLB 2	<u>O. glaberrima</u>	Sierra Leone
18	GLB BTO	<u>O. glaberrima</u>	Rice Research Station, Nigeria
21	LGS 101873	<u>O. longistaminata</u>	IRRI, Phillipines
22	GLB 101914	<u>O. glaberrima</u>	IRRI, Phillipines
23	GLB 3	<u>O. glaberrima</u>	Sierra Leone
24	GLB 101869	<u>O. glaberrima</u>	IRRI, Phillipines
25	GLB 101858	<u>O. glaberrima</u>	IRRI, Phillipines
26	SPT 100848	<u>O. spontanea</u>	IRRI, Phillipines

*Species names used in this study are as labelled on seeds received.

Results

From the correlation matrix, spikelet thickness was correlated with spikelet width, but it was negatively correlated with the colour of hairs on the spikelet, and the length of anther; and positively correlated with the presence of an appendage. Awn length and thickness; the length, density, colour, and distribution pattern of hairs on the spikelets were very strongly related to the shattering nature of the spikelet. These latter characters can be used as indicators of plants with close affinity to wild types.

The variation in the data was examined by means of a principal component analysis. This showed 32.92%, 14.02% and 11.87% of the variation to be due to the first three components, respectively (Table 3). From the weights of tests on components, the characters which contributed most of the variation in the first component were colour and distribution pattern of

Table 3

Importance of components and percent variance

Component	Weighting	Variance	Characters
1	32.92	32.92	Colour, distribution pattern of spikelet hairs, hairiness of awn
2	14.02	46.94	Thickness of spikelets, anther length, stigma colour
3	11.87	58.81	Length of spikelet and spikelet hairs, colour of grain, number of stigmas
4	10.76	69.58	
5	6.35	75.92	
6	5.90	81.82	
7	3.71	85.53	
8	3.31	88.84	
9	3.05	91.89	
10	2.07	93.96	

spikelet hairs, and the hairiness of awn. Thickness of spikelets, length of anthers, colour of stigmas and presence or absence of an appendage were more important in the second component.

Figure 1 shows a scatter diagram produced from the standardised data. Three groupings were differentiated (a) STF P 7237987 — quite isolated from the others, (b) all other wild annuals, and (c) *O. glaberrima* including an accession of *O. longistaminata*. When other characters were taken into account, by plotting the third axis (i.e. using length of spikelets, length of hairs on spikelets, colour of grain and number of stigmas) and the first axis additional groupings were formed and the relative positions of some of the accessions changed (Fig. 2). STF P 7237987 was still quite distinct from the others but came closer to *O. barthii*. STF 101050a associated with *O. barthii* and *O. spontanea* in Fig. 1. In Fig. 2. *O. barthii* separated out from this wild annual group.

Floret variation within cultivated types appeared to be mainly due to differences in length of anthers, colour of stigmas, and the thickness of spikelets whilst that within the wild annuals was due to the same differences, but the hairiness of the spikelets assumed greater significance.

The accessions were clustered from a Euclidean distance matrix, and presented in the form of a dendrogram (Fig. 3). The accession average technique illustrated is representative. At 50% phenon line 5 clusters emerged:

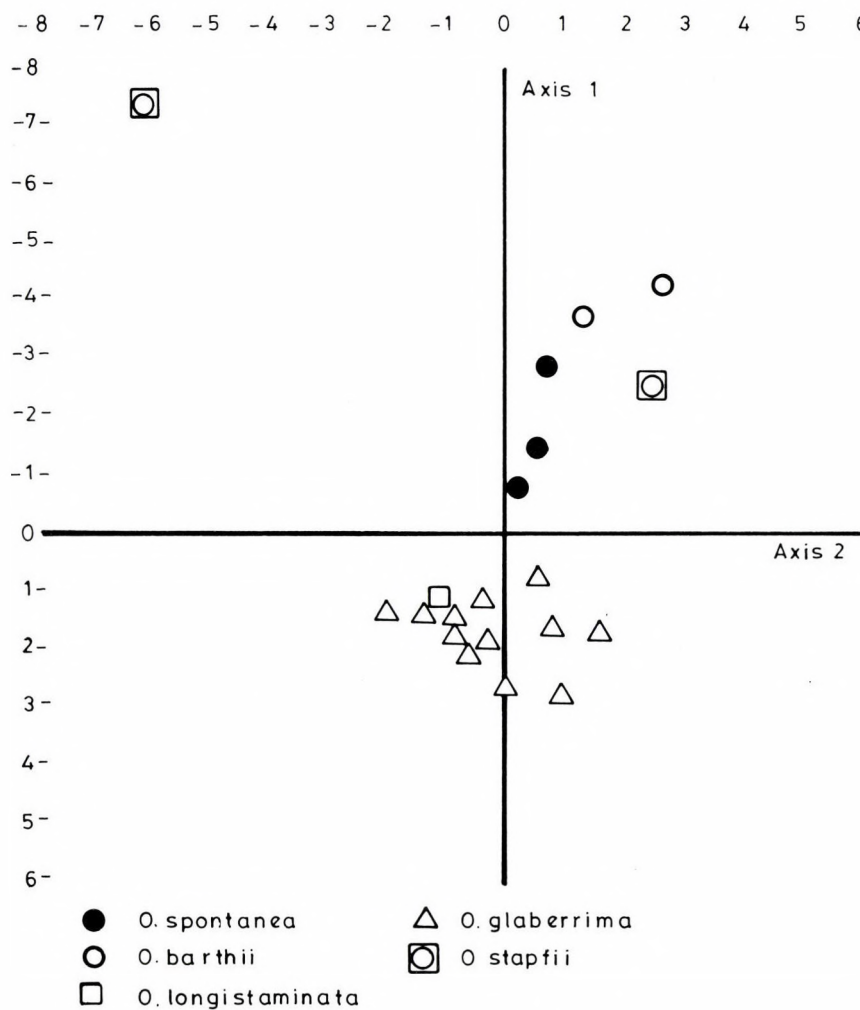


Fig. 1. Principal component ordination

- i) All the *O. glaberrima*, including the only *O. longistaminata*
- ii) *O. spontanea* (2)
- iii) *O. barthii* (2) and *O. spontanea* (1)
- iv) STF 101050a
- v) STF P 7237987

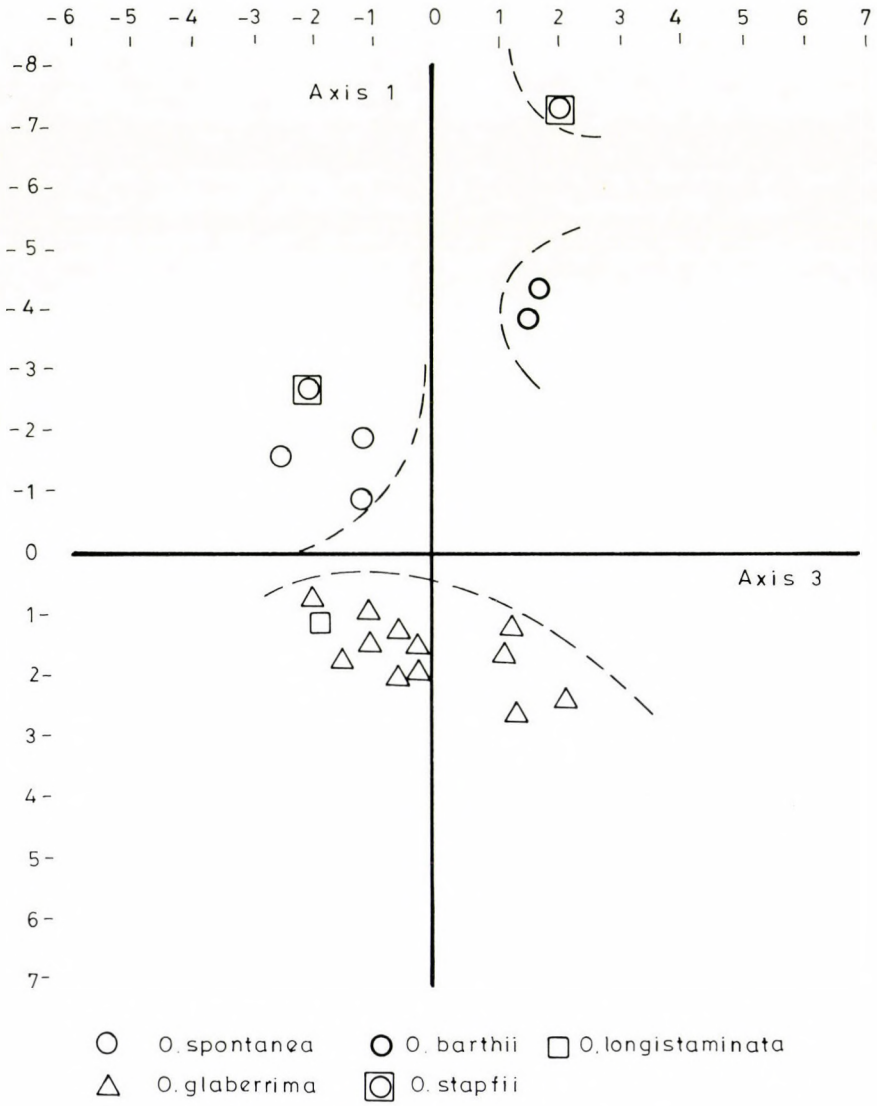


Fig. 2. Principal component ordination

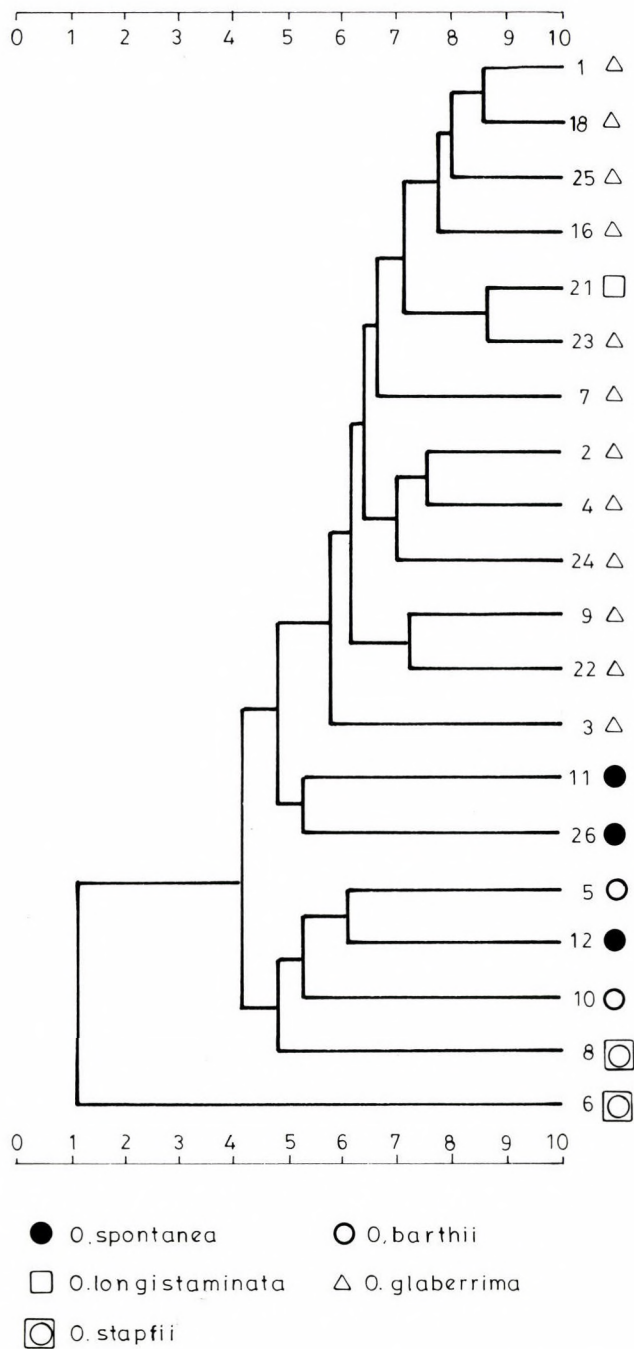


Fig. 3. Dendrogram. Clustering method by accession average from a Euclidean distance matrix

Discussion

There were more O. glaberrima accessions, but there was more uniformity among cultivated accessions than the fewer wild types. The O. barthii accessions were closer to the O. glaberrima. The O. stapfii accessions (2) varied widely, and STF P7237987 was quite distinct. These accessions of wild annuals seem to be part of a wider and genetically variable population.

The uniformity in reproductive morphology among the O. glaberrima accessions (12) compared to the O. barthii accessions (3) is due to artificial selection. Separation between wild annuals and cultivated O. glaberrima is based on a few characters -- colour and distribution pattern of spikelet hairs and hairiness of awns.

The close affinity between the annual wild African rices and O. spontanea is illustrated (Figs 1, 2, 3). It is not possible to distinguish between annual wild African rices and O. spontanea.

The O. longistaminata accession was observed to be intermediate between perennial O. longistaminata and O. glaberrima in the diagnostic features of ligule length and angle of ligule tip. This is further evidence to support introgressive hybridisation from the perennial African wild rice into other species in the complex (MORISHIMA et al. 1963; OKA and CHANG 1964; CHU and OKA 1970; DANIA OGBE and WILLIAMS 1978).

Some of the characters which separated the groups in these analyses have been studied genetically. Spikelet length has been studied by RAMIAH and RAO (1953), who found that shape of spikelet was associated with the colour and shattering nature of the spikelets; MORISHIMA et al. (1963) showed a very wide range in spikelet length, awn length as well as in apiculus hair length. Hairiness and spikelet length are characters used to distinguish O. barthii from O. glaberrima. RAMIAH and RAO (1953) also studied the genetic control of anther length. RICHHARIA et al. (1966) separated O. perennis from O. spontanea and O. sativa on anther length.

Summary

Characters of the reproductive phase succeeded in distinguishing cultivated from wild annual rices, the most important being the hairiness of spikelets. Variation within wild rices was far greater than that observed within the cultivated crop. It was not possible to distinguish African from Asiatic wild annual rices based on the characters observed.

Acknowledgement

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REFERENCES

- Chang, T. T. (1976): The origin, cultivation, dissemination and diversification of Asian and African rices. Euphytica 25: 425-441.
- Chu, Y. E., Oka, H. I. (1970): The genetic basis of crossing barrier between Oryza perennis subsp. barthii and its related taxa. Evolution 24: 135-144.
- Dania Ogbe, F. M., Williams, J. T. (1978): Evolution in indigenous West African rice. Econ. Bot. 32: 59-64.
- Hawkes, J. G. (1970): The taxonomy of cultivated plants. In: Frankel, O. H., Bennett, E. (eds): Genetic Resources in Plants: Their Exploration and Conservation. Blackwells, Oxford, pp.69-85.
- Hutchinson, J. B. (1965): Crop plant evolution: A general discussion. In: Hutchinson, J. B. (ed.): Crop Plant Evolution. Cambridge Univ. Press, pp.166-181.
- Morishima, H., Hinata, K., Oka, H. I. (1963): Comparison of modes of evolution of cultivated forms from two wild species, O. breviligulata and O. perennis. Evol. 17: 170-181.
- Navar, N. M. (1973): Origin and cytogenetics of rice. In: Caspari, E. W. (ed.): Advances in Genetics. Acad. Press, London, pp.153-293.
- Oka, H. I., Chang, W. T. (1964): Observations of cultivated and wild rice species in Africa. Report of Trip from Sierra Leone to Chad, 1963. Nat. Inst. of Genetics, Japan.
- Ramiah, K., Rao, M. B. V. N. (1953): Rice Breeding and Genetics. The Indian Council of Agriculture. Res. Scient. Monograph 3, No. 19.
- Richharia, R. H., Govindaswami, S., Gaosh, A. K. (1966): Introgression between O. perennis and O. sativa. A cause of variation in O. sativa var. spontanea (O. rufipogon) of Assam, India. Riso (Milano) 15: 183-191.

ALGUNAS NOTAS SOBRE LAS PLANTAS MEDICINALES, APLICADAS EN CUBA

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The experience of one year of investigations on the ethnobotanical studies realized in Cuba is offered in this paper through the revision of bibliography, survey to the Havana city citizens, and visits to different herbists. The main information recorded about the use and demand of some species and several recommendations on the importance of ethnobotanical studies are also given.

La flora de la Republica de Cuba posee 6375 especies (BORHIDI 1982, 1991), de ellas el 50% son endémicas. Sin embargo, la población solo aprovecha una pequeña parte de éstas.

Entre sus representantes encontramos especies de interés económico a las que la población da diversos usos de acuerdo a sus necesidades y gustos, lo que le da a esta un gran valor entre otros.

Las investigaciones etnobotánicas realizadas corroboran que el uso tradicional de las plantas medicinales, se mantiene en nuestros días como es el mas aceptado por la población. En la actualidad se observa una gran demanda de estas especies en las "yerberías" después de un largo período donde el uso de la medicina verde constituía un tabú, enmascarado por la interpretación mítica religiosa que se le daba al uso de las plantas medicinales, conscientes de la necesidad de rescatar nuestras tradiciones y educar a la población en este sentido, así como por el interés económico de sustituir importaciones y utilizar los recursos naturales renovables del país, que evitan los efectos secundarios que provocan algunos medicamentos sintéticos, las instituciones científicas desarrollan diferentes investigaciones encaminadas a resolver situaciones concretas en este campo.

En Cuba, la Etnomedicina siempre motivó a los científicos y ya desde el siglo XVIII se reportaron los primeros trabajos hechos por C. L. A. WALLERTON (1767—1770), toda esta ardua labor se compila en la obra del Dr. J. T. ROIG y MESA (1974).

En 1973 con la fundación de la Estación Experimental de Plantas Medicinales se continúan las investigaciones sobre este tomo en Cuba, destacándose los estudios realizados por FUENTES et al. (1980, 1982), que incluyen análisis fitoquímicos, genéticos, cultivos, etc. de plantas principalmente cultivadas.

Las especies medicinales utilizadas tradicionalmente en Cuba alcanzan la cifra de 696, agrupadas en 132 familias y 441 géneros (FUENTES et al. 1986).

El cultivo de las plantas medicinales en Cuba, al igual que en otros países se realiza por parte de la población en jardines y patios, cerca de las viviendas para utilizarlas preferiblemente frescas. Además se utilizan muchas plantas silvestres de lugares yermos y vegetación secundaria principalmente.

El conocimiento del uso de las plantas medicinales, parece ser general, aunque se destacan los ancianos "conocedores" cuyas edades oscilan entre 70 y 90 años, y aquellos grupos poblacionales que viven en zonas rurales que mantienen estas costumbres transmitidas de generación en generación y conocen específicamente cuando aplican y a que dolencia determinada "hierba".

En sentido general se ve un incremento en los últimos años en el número de usuarios que reclaman "hierbas" que conocieron sus usos a través de sus antecesores divulgación nacional o en busca de la recomendación que el "yerbero" da para curar su afección. En esto se ven períodos de "auge" en el uso de determinadas especies vegetales, tal es el caso del "anamú" (Petiveria alliacea), o el extracto del tallo del "plátano" (Musa paradisiaca), utilizadas para combatir el cáncer, esto provocó una explosión en su utilización que sugirió investigaciones profundas en el campo científico. Recientemente la "sábila" (Aloe barbadensis y Aloe sp.) ha alcanzado gran notoriedad tanto por los variados usos populares, como por los aciertos científicos, en ella encontrados, destacados como: regeneradora de tejidos (en úlceras crónicas, hemorroides, afecciones de la piel, heridas etc.), afecciones bronquiales y cosméticos entre otros. Así mismo se incluye el "té de riñón" (Orthosiphon stamineus), de gran demanda para tratar afecciones renales, introducida en los últimos años, alcanzando éxitos en su aclimatación y cultivo.

La preparación de las plantas medicinales es simple y la población cubana prefiere utilizarlas fresca, lo que parece ser influido por la costumbre, aunque también la utilizan secas para preparar decocciones o in-

fusiones que puedan beberse, inhalarse, o aplicarse externamente en fricciones o como cataplasma, en otras ocasiones se mastica directamente la planta verde, dependiendo de la dolencia a tratar. Para algunas especies vegetales, se siguen ciertas condiciones específicas de selección o procedimiento que pudieran determinar sus atributos, por ejemplo para preparar uno de los jarabes que "cura" el asma se necesita que esté expuesto durante varios días al sol.

Las enfermedades mas comunes tratadas en la actualidad con "hierbas medicinales" son: afecciones renales, bronquiales, estomacales, dérmicas, diabéticas y reumáticas.

Aunque en Cuba siempre existieron personas dedicadas a la venta de plantas con fines medicinales y folclóricas, en lugares denominados "yerberías" (tradicional comercio de plantas), hubo un período en que decayó esta actividad producto de la industrialización de país, de los avances de la ciencia y la técnica y por el sentido oscurantista que se le atribuyo a la misma, sin embargo en la actualidad dado el auge que toma por necesidades concretas del país en el campo de la ciencia, el desarrollo de la medicina verde, se mantienen estos establecimientos en distintas provincias donde acude la población en busca de diferentes plantas.

Entre las especies que han mantenido tradicionalmente sus usos y acepciones se destaca: "tilo" (Justicia pectoralis), "manzanilla" (Matricaria chamomilla), "verbena" (Stachytarpheta jamaicensis), "guira" (Crescentia cujete), "albahaca" (Ocimum sp.), "escoba amarga" (Parthenium hysterophorus), "mastuerzo" (Lepidium virginicum), "guizaso de caballo" (Xanthium strumarium), "mejorana" (Majorana hortensis).

Hay especies que actualmente se utilizan para combatir diversas afecciones que antiguamente eran específicas para una determinada dolencia, entre ellas tenemos:

Tabla 1

Nombre vulgar	Nombre científico	Uso tradicional	Uso actual
Sábila	<u>Aloe barbadensis</u>	enfermedades del hígado	regenerador de tejido
Reseda	<u>Lawsonia alba</u>	folclórico	sedante astringente dolor de estomago
Vicaria	<u>Vinca rosea</u>	colirio	para tratar la leucemia

Existen algunas especies que actualmente se usan muy poco, como la "agüedita" (Picramnia pentandra), "yamaqua" (Guarea guidonia), "cuajani" (Prunus occidentalis), muy utilizadas por la población y nuestros mambises durante la guerra de independencia, la primera para tratar las fiebres altas en sustitución de la Quina, (Cinchona spp.) la segunda para controlar las hemorragias, y la tercera para las afecciones bronquiales.

Entre las plantas medicinales intercambiables ("confundidas") con otras con porte similar, por la población se encuentran:

Tabla 2

Nombre vulgar	Nombre científico	Observaciones
Paraíso francés	<u>Moringa oleifera</u>	En ocasiones se da como sedante por tilo (<u>Justicia pectoralis</u>)
Platanillo de Cuba	<u>Piper ossanum</u>	El mismo nombre vulgar se utiliza para las especies de <u>Canna</u> por lo que se ha utilizado en lugar de <u>Piper</u>
Manzanilla	<u>Matricaria chamomilla</u>	Como manzanilla lo mismo se utiliza la antes citada que <u>Isocarpha</u> sp. y <u>Phania matricarioides</u>
Brasilete	<u>Caesalpinia bahamensis</u>	Estas especies son muy utilizadas para afecciones renales y a veces para diabetes a menudo los yerberos proponen una por otra y ha sido confundida con el "Palo campeche" (<u>Haematoxylum campechianum</u>)
Brasil	<u>Caesalpinia vesicaria</u>	

Por último relacionamos las plantas medicinales de uso tradicional:

Tabla 3

Nombre vulgar	Nombre científico	Afecciones
Caña mexicana	<u>Costus speciosus</u>	renales
Mastuerzo	<u>Lepidium virginicum</u>	
Nitro	<u>Boldoa purpurascens</u>	
Té de riñón	<u>Orthosiphon stamineus</u>	
Chichicate	<u>Urera baccifera</u>	bronquiales
Caña santa	<u>Cymbopogon citratus</u>	
Orégano francés	<u>Coleus amboinicus</u>	
Majagua	<u>Hibiscus elatus</u>	
Güira	<u>Crescentia cujete</u>	reumáticas
Platanillo de Cuba	<u>Piper ossanum</u>	
Caisimón	<u>Potomorphe umbellata</u>	
Eucalipto	<u>Eucalyptus</u> sp.	

Tabla 3 (contd.)

Nombre vulgar	Nombre científico	Afecciones
Cúrbana	<u>Canella winterana</u>	
Albahaca morada	<u>Ocimum sanctum</u>	
Palo de caja	<u>Allophylus cominia</u>	
Tuatua	<u>Jatropha gossypifolia</u>	diabéticas
Escoba amarga	<u>Parthenium hysterophorus</u>	dérmicas
Ponasí	<u>Hamelia patens</u>	
Manzanilla	<u>Isocarpa</u> sp.	
Verbena	<u>Stachytarpheta jamaicensis</u>	

REFERENCIAS

- Barrera, H. (1979): La etnobotánica: Tres puntos de vista y una perspectiva. INIREB, Xalapa, Veracruz, Mexico, p. 30.
- Borhidi, A. (1982): Florengeschichte der Antillen im Licht der Kontinentalverschiebungstheorie. Stapfia 10: 235—241.
- Borhidi, A. (1991): Phytogeography and Vegetation Ecology of Cuba. Akadémiai Kiadó, Budapest, p. 858.
- Caballero, P. (1979): Perspectivas para el quehacer etnobotánico en México. En: La Etnobotánica: Tres puntos de vista y una perspectiva. INIREB, México, p. 27—30. /Citado por H. Barrera, 1979./
- Fuentes, V., Granda, M., Xiques, X., Gutiérrez, C. (1986): Encuesta sobre la utilización popular de plantas medicinales. Memorias. Séptimo Seminario Científico del CNIC, p. 281.
- Fuentes, V., Granda, M. (1982): Estudio sobre la medicina tradicional en Cuba. I. Rev. Plantas Medicinales, 2: 25—46.
- Gómez de la Maza, M. (1889): Ensayo de Farmacofitología cubana. La Habana, Propaganda Literaria.
- Grossourdy, R. de (1864): Médico botánico criollo. (Brachet, F., ed.), París, 4 tomos, pp. 426, 512, 416, 510.
- Hernández, J. (1976): El concepto de Etnobotánica en México. En: La Etnobotánica: Tres puntos de vista y una perspectiva. INIREB, México, p. 13—19. /Citado por H. Barrera, 1979./
- Roig, J. T. (1974): Plantas medicinales, aromáticas o venenosas de Cuba. Cien. y Técn., Instituto del Libro, La Habana, p. 949.
- Róque, A., Cabrera, C., Pérez, A. (1983): La medicina popular en el Municipio Palmira, provincia Cienfuegos. p. 30.
- Toledo, V. (1978): Estudio botánico y ecológico de la región del río Uxpanapa, Veracruz no. 7. El uso múltiple de la selva basado en el conocimiento tradicional. México, Biotica, vol. 4, 2: 103—105.
- Wallerton, C. L. (1767): Tratité explicatif de berbier des plantes medicinales recueillées dans un voyage à la nouvelle Espagne et à île de Cuba. Nancy, vol. 3, 1767—1770.

BOOK REVIEWS

ed.: K. T. KISS

JORHIDI, A.: *Phytogeography and Vegetation Ecology of Cuba*. — Akadémiai Kiadó, Budapest, 1991. 858 pp. 380 Figs, 16 colour tables, 143 tables, 1 map. 74.- USD

The first complete and comprehensive geobotanical survey of the vegetation of a Latin-American country has been presented in this marvellous book. I would propose it as a model of this kind in the tropical and subtropical areas. Spending more than 6 years with field and herbarium work in Cuba the author had fair opportunities to study the composition and distribution of the main vegetation types and phytosociological units of the whole country.

This is the first example in the botanical literature to demonstrate how to investigate a tropical flora and vegetation parallelly by using various vegetation-studying methods. One can see the first attempt to present how to overturn the Richard's transects into the Braun-Blanquet's plots and how to applicate a classic Middle European method of Braun-Blanquet to a survey of tropical vegetation. The pioneering initiative of the author is already followed by more researchers; P. GUTTE and G. MÜLLER in Peru; by VAN CLEEF and collaborators in the Ecoandes program and VAN DER MAAREL in studying the coastal vegetation of Mexico. The book contains the computer analysis of 40 units of tropical forest and shrub vegetation based on exact phytosociological data tables containing more than 2000 species. An outstanding result of this work is a concise new theory for the flora evolution of the Antilles based on the author's comparative flora analyses, original taxonomic works and the results of the plate tectonic. It is not an exaggeration to say this monumental work will be an excellent starting-point of all the further phytogeographical and vegetation studies in the Caribbean.

After the introduction, the enumeration of scientific preliminaries, approaches objectives, conceptual and methodological details the book is divided in 6 great parts.

The bioclimatological and edaphical fundamentals are presented in the first two parts, the phytogeographical fundamentals and division of Cuba in the 3rd and 4th parts, the main vegetation types in the 5th part and the classification of Cuban plant communities in the 6th part.

1. Bioclimatological fundamentals

The first part gives a short concise view about the main geological, geographical and bioclimatological characteristics of Cuba including oro- and hydrography, distribution of temperature, atmospheric currents and precipitation; the phytogeographic roles of the cyclones and hurricanes. This is the first comprehensive bioclimatical study of the country using correctly the GAUSSEN's terminology and WALTER's graphical method, giving the description and distribution of 12 bioclimatic types and their correlation to climax vegetation belts. It is remarkable that a new bioclimatic type is also described, which is especially correlated to the sclerophyllous tropical vegetation. Some new interesting thoughts about dynamic climate-vegetation relationships in the Caribbean including some critical remarks about the HOLDRIDGE's life zone concept are deeply discussed as well.

2. Relationships between the soil and vegetation in Cuba

An unsurpassed compendium of the tropical soil sciences with the main fundamentals is given in order to conceive the complicate relationships between the soil and vegetation in tropical territories. It begins with the brief survey of the classifications of Cuban soils. A new soil classification of ecological viewpoint is developed considering the influence of the special climatic effects here. The role of serpentines is one of the most special and exciting problem concerning the tropical soil-formations in Cuba. Every locality of diverse

serpentine rock-formation have a characteristic, mostly endemic flora, a special successional series of sclerophyllous vegetation units ending in special serpentine climax. The climate and soil conditions of a long-term tropical succession are also modelled and discussed.

The ecology and origin of Cuban savannas is a highly important question, being the 92% of the Cuban territory covered by grasslands and agricultural fields. Taking into account both BENNET's and BEARD's classic savanna concepts a reconsideration of Cuban grasslands is explained based on their physiognomy, composition and genesis.

3. Fundamentals of the phytogeography of Cuba

The fundament of explaining the phytogeography of this insular country is the accurate life form analysis treating critically all the recently used life-form systems both for the spermatophytes and pteridophytes living in Cuba. The studies on life-form vicariancy, on growth and chorological types and on special phytogeographic and ecological features of the flora like microphyllia, micranthia, vulnerability, the inversion of floristic elements and vegetation types, the dominance of endemics produced a brilliant synthesis of the phytogeographic characterisation of the flora of Cuba.

4. The phytogeographical subdivision of Cuba

Improving of GOOD's geobotanical regionalization of the Caribbean phytogeographic region the authors, with the important co-authorship of O. MUNIZ, proposed a new phytogeographical subdivision for the Caribbean and the Cuban Flora-Province dividing the country into 3 sub-provinces: Western-, Central- and Eastern-Cuba, 9 sectors and 36 districts, characterizing each units by enumerating sectorial and local endemics characteristic vegetation types, and presenting numerous flora maps and vegetation transects.

5. The vegetation map of Cuba

After exposing the fundamental informations about the general ecological and phytogeographical characteristics, starting from the 5th part the author presents detailed results of his field studies. In this part he characterizes the vegetation types studied by an improved application of Braun-Blanquet's method as a new approach in studying the New World vegetation. 25 vegetation types of forests and shrub-woods in 7 groups, 9 of savannas and grasslands, 4 of freshwater — and 3 of coastal vegetations are presented and accurately analysed. The list is completed by a computer oriented classification of the major forest types of Cuba contributed by Z. SZÖCS, and by the unique vegetation map of Cuba joined to the cover of the book. 40 forest and shrubwood communities containing more than 2000 taxa with 38 characteristics of each taxa were used by the new computer analyses. The results are in a good agreement with the ones of other classical investigations, confirming the author's starting hypotheses on the development and migration of the endemic flora, and suggests that the vegetation types in Cuba are not only typical physiognomic categories but at the same time they are also phytosociological and ecological units and in many cases centres of flora development.

6. Systematic survey of plant communities

This part makes almost half of the book, and gives a detailed analysis of 56 orders in 27 classes described by the application of Braun-Blanquet's system. The first part of the Appendix belongs to this part: exact cenological tables of the investigated communities. In addition, the most characteristic tables are presented in the text itself of the relating units. The description of the order includes its short characterization, the main character-species, the inquired subdivisions and the description of the main associations illustrated with some prominent black and white photos.

The value of the book grows by an exhaustive literature. The subject index and the register of Latin names enumerates the names of vegetation units and of mentioned species, respectively. 16 splendid colour photos of the most characteristic Cuban vegetation types, the excellent map of the natural potential vegetation in a scale of 1:1125000 — made by the author and O. MUNIZ, the outstanding Cuban biogeographer — are attached to the end of the book.

I am convinced that this wonderful book may be an indispensable basic source of information not only for the Cuban naturalists but for all educated people, teachers and scientists working or interesting in any kind of tropical vegetation sciences.

Z. KERESZTY

NATHO, G., MÜLLER, C., SCHMIDT, H.: Morphologie und Systematik der Pflanzen I—II. In Uni-Taschenbücher für Wissenschaft — Wörterbücher der Biologie. — Fischer Verlag, Jena, 1990. 852 pp.

Nach einer langen Entstehungs- und Entwicklungszeit, nach einer völligen Veränderung kann jetzt der gütige Leser diesen Band endlich in Hand nehmen. Die bis 1959 im wesentlichen abgeschlossene Manuscriptbearbeitung als "Systematik der Pflanzen", etwa 1000 Stichwörter umfassend, wird in den folgenden Jahren infolge der objektiv bedingten widrigen Umstände immer wieder den Abschluss der Endredaktion verhindert und zu guter Letzt um ein weiteres Bündel von Stichwortbearbeitungen bereichert, deren redaktionelle Durchsicht weitere Jahre verlangt hat. Im Jahre 1971 wurde dann die rigorose Dezentralisierung des Wörterbuch-Vorhabens verkündet. So musste das Manuscript für ein "Wörterbuch der Allgemeinen und Speziellen Botanik" völlig übergearbeitet werden. Die Systematik wurde mit der Morphologie zusammengekoppelt, die niederen Pflanzen und auch die Erweiterung selbst zeigten — sicherlich berechtigt — separatistische Tendenzen und erhielten neue Bearbeiter. Die Schwierigkeiten wurden aber auch durch weiteren Vorfälle vergrößert. Der langjährige Betreuer des Bandes, Prof. H. BORRISS ist im Jahre 1985 verstorben. Die lange Entstehungszeit machte die neuerliche Bearbeitung einzelner Manuscriptteile erforderlich. Die Herausgeber haben sich letztlich noch die Einbeziehung der Pilze entschieden. Die in die Serie "Mikrobiologie" zugeplante Cytomorphologie und die Kryptogamen sollten jetzt auch zusammengefasst werden. Dieses endgültige zweibändige Wörterbuch enthält so etwa 4000 Stichwörter zur Systematik und Morphologie der Algen, Pilze, Moose sowie der Farn- und Blütenpflanzen. Jedes Stichwort wird ausführlich und doch nur "im Kratzen" erläutert und durch Querverweise in einen grösseren Zusammenhang gestellt. Die Textaussage wird durch zahlreiche Strichzeichnungen vertieft.

Nur durch die Vorstellung der oben Vorhergehenden kann die Mängel des Werkes einsehen und verzeihen. Der Herausgeber hätte den Unsinn einer solchen Vermischung schon am Anfang wahrnehmen müssen. Eine relative Vollständigkeit wird von jedem wissenschaftlichen Wörterbuch verlangt, eine Volksausgabe dieser Art ist ja überhaupt kein glücklicher Einfall. Diese Vollständigkeit kann aber in einem so ungeheuer grossen Bereich einfach nicht durchgeführt werden. Auch die Unverhältnismässigkeit der Beschreibungen ist der mehrmaligen Durchbearbeitung zu verdanken. Wenn man z.B. die Beschreibung von Antheriden (I, 36) oder sogar von Anthocerotopsida (I, 37) und im allgemeinen die von Kryptogamen sieht, bemängelt sie rechtmässig z.B. die verschiedenen Sorten des Döldchens (I, 234) oder etwas mehr von Genzentrum (I, 316). Um die Platzmöglichkeit besser auszunützen, die Autoren haben viele zusammengehörigen Fachausdrücke in die Beschreibung eines Stichwortes gruppiert. Die wahrliche Lösung des ganzen Problems wäre die Systematik und die Morphologie völlig separat zu verhandeln. Es muss trotzdem bestätigt werden, dass die Beschreibungen der einzelnen Stichwörter richtig und zeitgemäss sind. Die Autoren haben nach der Verwendung der neuesten wissenschaftlichen Resultaten gestrebt. Bis die Systematik ziemlich gut repräsentiert ist, könnte die Morphologie etwas ausführlicher dargestellt werden. Einige Stichwörter, wie z.B. die zu "di-" (didynamie, digenie, dignie) sind ganz und gar ausgeblieben, obwohl sie ebenso wichtig und bekannt sind als die anderen. Die kleinere Begleitzeichnungen und Abbildungen sind ausgezeichnet hinpassend, von den besten bezüglichen Werken ausgewählt. Die ganzseitigen Abbildungen sind aber überflüssig, sie nehmen viel Platz ein. Statt dessen wäre es besser gewesen, das Wörterbuch mit den mangelnden Stichwörtern zu ergänzen. Obwohl ich dieses Werk mit den kürzeren Beschreibungen, mit mehr winzigen Begleitzeichnungen und mit den mangelnden Grundbegriffen ergänzt für ein ideales Erklärungs-Wörterbuch halte, ist es auch in dieser Form sowohl den Studenten und Dozenten, als auch allen Pflanzenfreunden als eine gut benützbare Quelle vorzuschlagen.

Z. KERESZTY

CULLEN, J., DAVIS, P. H.: The identification of flowering plant families. — 3rd ed. Cambridge University Press, 1989. 133 pp.

The second edition of this practical taxonomic pocket-book was sold out over the past decade, and besides, in the light of new statements of many changes in taxonomic thinking and in public attitude to plants and wildlife in general, a completely revised third edition in need, which is intended for all those with a practical interest in plant identification and who wish to understand the scientific basis of the identification procedure. On the other hand, there have been some increasing public awareness of the need for conservation as well which has led to a renewed interest in plants. This interest should have been satisfied in part by cheap, small, well-illustrated popular books on plants available today. The authors therefore revised the 2nd edition using the latest results, the modern terminology based on the construction of the floral morphology by means of Miss R. M. SMITH's excellent illustrations and with good practical sense, in the interest of developing a way of easy and quick identification. This work provided enables to the first step of the identification of 285 flowering plant families, normally found either wild or in cultivation in northern temperate regions. Only few tropical families which rarely flower in cultivation have been excluded. The main changes from the second edition are the adoption of the ENGLER—PRANTL taxonomic system, since it still seems to be the best one for general purposes; the new keys have been modified to take account of errors and enlarged to provide more guidance; the section "Further identification" has been completely rewritten; the terminology changes according to the full knowledge of the new facts.

Following the authors' Preface and Introduction the work is divided into 7 chapters. In the first chapter the most important botanical terms are described and very persuasive illustrated. In order to make an accurate differentiation possible in floral morphology the attributes hypo-, epi- and perigynous are used for the internal arrangement of the flower as well, with respect to that between the ovary and the various floral parts taken in relation to one another, thus both the perigonium and the petal or the stamen can be of epi-, hypo- or perigynous arrangement. The next two chapters deal with the methods in preparing of the plant to the identification and the using of the keys. This follow two large chapters; the keys and the family descriptions. Since the mean keys are built on the flower arrangement, this key-system is not suitable for the identification in the vegetative and native state. Nevertheless, it is a great help for specialists and plant-loving non-professionals that the whole plant kingdom narrows down to 14 great groups already at the head keys, then, by branching them off further, the user of the book can very quickly recognize the family. With the help of the short characterizations of the families the possibility of "reversed determination" also can arise, the accuracy of our identification can be tested. This chapter involves the short practical characterizations of the orders as well. The high didactical value of the book manifests from the last chapter containing help to facilitate the further identification with the aid of the rich annotated bibliography completing by a capable morphological glossary and the index of the family names.

This small pocket-book is intended for all plant-loving people, whether they are botanists, gardeners or otherwise involved with plants, either as students, professionally or as a hobby. It will serve — like the former edition — as an up-to-date manual for them.

Z. KERESZTY

GLEDHILL, D.: The names of plants. — 2nd ed. Cambridge University Press, 1989. 202 pp.

The author, one of leading personalities of the morphology school in Bristol University, have aimed at producing an interesting pocket-guidebook for the amateur gardener and for young botanists to present the bases and the change of the plant-naming. The first edition in 1985 had a shorter discussion over the nature, the size and the solution of the naming-problems.

Following this it deals with the rules of botanical nomenclature and with the usage of the botanical terminology. A particular merit of the book is that includes both the common and botanical names, both their abbreviated garden forms. The major part of the book consists of the alphabetical list of the Latin plant names both of the genus and the species, including the frequent etymons of epithets e.g. *acmo-*, *adeno-*, *-cillus*, *-codon* etc., and their meanings in English, which continued to grow by the year but which could never be complete.

For this second edition a number of changes and corrections in both parts have been made. The author has attempted to keep the first part acceptable to the amateur gardener by resisting a temptation to make it a definitive guide to the International Code of Botanical Nomenclature. Revision has allowed the inclusion of a brief comment on both synonymous and illegitimate botanical names and reference to recent attempts to accommodate the various traits and interests in the naming and names of cultivated plants. This small popular-edition may be used successfully by dealing with the botanical nomenclature, but it is considered as an excellent botanical dictionary for the foreign botanists and gardeners as well. Students who are interested in this theme can use the book with many benefit, too.

Z. KERESZTY

WEBERLING, F.: *Morphology of flowers and inflorescences*. — Cambridge University Press, 1989. 405 pp.

The well-known German book (1981) providing an excellent survey of the higher plant morphology can be welcomed in this English translation. It is based on W. TROLL's and E. STRASBURGER's works, containing the numerous and varied results of the scientific development of today. In the present work the author has attempted to combine the results of earlier and more recent individual studies on the anatomy of flowers and floral organ into an excellent synthesis. The flower morphology is the most interesting field within the morphology and is of the most importance for flowering plant systematics.

The book is divided into 3 main parts. The first part coming to the half of the whole text includes the basic and general information of the floral morphology. To the best understanding of the text a practical short glossary compiled from the most frequent terms is preceded. The text gives a key to the different directions in which the further literature leads by means of particular citations and references. In the selection of material not pursued perfection but tried to allow access to the different areas of plant morphology has been chosen. In the second part the morphology of the inflorescence is discussed. There is a short and comprehensive description of this characterization according to the investigation of W. TROLL and certain of his students in the past decades, made use of his splendid illustrations and photograph collection. A peculiar merit of this book, which looks best in this part, is the functional point of view, without this no satisfactory explanation of morphological phenomena can be given. In the third part the pollination-morphology, the interrelationship between flowers and pollinators, the systematics of the fruits and the dispersal mechanisms are presented.

The very well-illustrated book is supplemented by an abundant bibliography and by an index of scientific names and general ones.

In conclusion this book is written in a simple, straightforward, lucid and comprehensible style. It is of interest to all practising botanists and is proposed in particular for all the schools and institutes dealing with the instruction of biology.

Z. KERESZTY

DELCOURT, P. A., DELCOURT, H. R.: Long-term Forest Dynamics of the Temperate Zone. Ecological Studies Analysis and Synthesis. Vol. 63. — Springer Verlag, New York, Berlin, Heidelberg, London, Paris, Tokyo, 1987. 439 pp.

Volume 63 of Ecological Studies represents a comprehensive and quantitative examination of the paleoecological evidence for forest community development in the temperate zone of eastern north America since the last glacial maximum 20000 years ago.

Computerized fossil-pollen data of 162 radiocarbon-dated paleoecological sites were used. 19 major (dominant, subdominant and characteristic) tree taxa were selected to estimate the major trends in change of vegetation pattern in the past.

In the last chapter the authors summarized the Late-Quaternary vegetational and climatic history of Europe too, on the basis of the work of HUNTLEY and BIRKS. They compiled fossil-pollen data from 843 paleoecological sites throughout Europe. They mapped the most important 15 taxa and made a reconstruction of European vegetational history through the past 13000 years.

Finally the authors made a comparison of the major migration routes between the European and eastern north American key tree taxa, which is a unique interesting and very useful study for the European paleobotanists.

There is no doubt that this book in this respect is a pioneer work.

It is a pity that neither HUNTLEY and BIRKS nor the authors of this book did not accept the Hungarian paleobotanical results even if Hungary is included in the region they studied.

To summarize it, the whole book gives valuable information about the Late-Quaternary migrational strategies of most important tree species.

M. JÁRAI-KOMLÓDI

THROWER, S. L.: Hong Kong Lichens. — Urban Council. 1988. 193 pp. UC 30328. 40.- USD

I was very enthusiastic to read about the publication of the "Hong Kong Lichens" in the International Lichenological Newsletter. When I first dipped into the book it was a pleasant surprise that besides the species list and the keys for identification, a great number of marvellous colour photographs taken either in the nature or in the herbarium are included. (Although in some cases of rather poor quality.)

The book covers very important and useful general parts (Structure of the lichen, Reproduction, Aids to identification, Glossary and figures), and special parts (Distribution and ecology, Keys for identification, richly and beautifully illustrated Descriptions) treating the lichen of Hong Kong.

The number of species listed (200) is comparable to that of the Hong Kong-size areas surrounding Budapest. In spite of the high level of human impact on their ecological conditions, the "ever-increasing disappearance of their habitat", the "many centuries of deforestation" it is quite a nice number. Beyond the surviving fruticose and foliose lichens a large proportion of the less sensitive crustose lichens is included. It is remarkable that some critical taxonomic groups (e.g., *Arthoniaceae*, *Graphidaceae*) are especially well represented. The difficulties in identification of tropical lichens are shown by the fact that only 146 species (from the total of 200) were possible to name more or less precisely even by the help of specialists. Studying lichens of a little known area it is often essential to know the source of the name. The author's method is worth being followed as she indicated if the species 1) was named by a monographer, 2) by her (using herbarium specimens from abroad for comparison), 3) compares with (cf.) or related to (rel.) a specimen, or 4) probably an undescribed, new species (only family or genus name is given).

The structure of the book as a whole is practical to the user. The author's remarks concerning the status, confusable species and substrate are valuable. Among the references books of general information and interest, keys and floras useful in Hong Kong, and monographs are mentioned. Nevertheless, this list could have included some more recent works, too, e.g., GALLOWAY's (1985) Flora of New Zealand lichens.

Let the author's attitude to lichens (as the "Cinderellas of Hong Kong plant world") and nature conservancy be a model for amateur botanists and collectors who should rather "leave only foot-prints, take only photographs". Since the tropical Asian regions are the less studied of the world lichenologically, this colour guide renders a very good service not only to students of any age, but also to lichenologist specialists.

E. FARKAS

HINDÁK, F.: Studies on the Chlorococcal algae (Chlorophyceae) V. — VEDA Publishing House of the Slovak Academy of Sciences, Bratislava, 1990. 225 pp.

The reader can get acquainted with a newer volume of HINDÁK's series about chlorococcal algae. On the aim of the book we can get the most correct information from its introduction. "As in the preceding volumes of the Studies we would like, in this case, too, to point primarily at the framework of the variation of significant diagnostic features of investigated algae, both under natural and laboratory conditions. However, limited possibilities especially in the area of laboratory cultivation have allowed to keep track only of a certain part of this variability. It is this aspect from which the results presented and the taxonomic conclusions are to be assessed. A certain disadvantage of this examination is that it relies on knowledge gained from studies made with the light microscope and that no data of our own are available on the fine structure of cells. Hence in this area we lean upon literary data published especially in recent years."

After a short material and methods chapter, the book reports on 7 genera (Didymocystis, Pseudodidymocystis, Didymogenes, Tetrademus, Dimorphococcus, Enallax, Scenedesmus) with 54 species and varieties. Among these 4 are new combinations. In the book 107 plates with more than 1000 well-made algal drawings are given. After the genera described we can find some remarks on the studies on the Chlorococcal algae IV, mainly about some nomenclatural errors. The book contains 91 references, an English, Slovakian and Russian summary and an object index with italics the name of synonyms or mentioned incidentally.

HINDÁK's book is warmly recommended to algologists and hydrobiologists who deal with algae identifications. The book is very useful to help their work.

É. ÁCS

CALLOW, J. A. (ed.): Advances in Botanical Research. Vol. 14. — Academic Press, London, San Diego, New York, Boston, Sydney, Tokyo, Toronto, 1987. 198 pp.

This volume consists of three articles. Each of them is an exact and circumspect summary about its own field.

The first one is written by ELLIS and ROBINSON. In this article the authors discuss the basic principles concerning protein targeting. In their opinion "A central problem for cell biologists is to unravel the mechanisms which ensure that proteins are directed from their site of synthesis to the site where they function. The term 'targeting' is used to indicate the directional nature of these mechanisms." Much of the information about targeting is derived from animal and microbial research and only a few experiment has been carried out with plant cells, but on the bases of our recent knowledge it seems that some principles of the mechanisms of targeting are universal.

The aim of the second article which is written by GRAY is "to consider our knowledge of the control of isoprenoid biosynthesis in higher plants, particularly with respect to the properties and subcellular locations of the enzymes involved". In higher plants a lot of isoprenoid compounds play vital roles in the metabolism and development of the plant, including defence, structural components of membranes, electron transport molecules, hormones, intermediates in polysaccharide synthesis and many other activities. Thus GRAY recommends his study:

"In this article the experimental evidence for postulated regulatory mechanisms will be critically assessed and an attempt will be made to provide a conceptual framework for further experimentation."

GINZBURG's article deals with a unicellular, photosynthetic green alga, *Dunaliella*, which is able to grow over wide ranges of pH and temperature and to be accommodating to changes in composition of the growth medium. The purpose of this chapter according to the author is "to summarize and analyse the often conflicting data on *Dunaliella* gathered during the past 30 years, to compare and contrast *Dunaliella* with other green algae, and to further the understanding of the adaptation of *Dunaliella* to high salt concentrations."

Having looked over the content of this book it can be recommended to botanists, algologists working in cell biology, physiology or biochemistry. The articles are very valuable summaries about these interesting themes and they are indispensable for further investigations on their fields.

B. PAPP

FUKUYO, Y., TAKANO, H., CHIHARA, M., MATSUOKA, K. (eds): Red Tide Organisms in Japan — An Illustrated Taxonomic Guide. — Uchida Rokakuho, Tokyo, 1990. 430 pp.

FUKUYO and his co-workers edited an unique book using the work of 23 scientists. It is unique, because hardly any separate publication has been published on red tide organisms although an increasing number of articles has been written on their biology and the problems caused by these organisms.

The book is so well illustrated that it can even be used as a taxonomical work though it has not been written with that purpose.

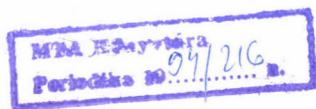
Citing the editors preface "In this book, we include not only causative organisms of red tides but also toxic species and organisms which are associated with other dominant species in red tides occurring along Japanese and Southeast Asian coastal waters. We also include some freshwater red-tide organisms in this book." That is the reason why red-tide organisms abundant in other areas/waters are not listed here. Obviously, the book also describes a number of species which are not only red-tide organisms. A couple of species are known from the usual plankton of "common waters" others are common or abundant/dominant in water blooms.

8 Cyanophyceae, 2 Cryptophyceae, 70 Dinophyceae, 85 Bacillariophyceae, 9 Raphidophyceae, 6 Chrysophyceae, 4 Haptophyceae, 8 Euglenophyceae, 5 Prasinophyceae, 1 Chlorophyceae and 2 Protozoa species are listed in the book determining that it is basically an algological work.

Without any taxonomical keys the authors characterize the species also by enclosing lightmicroscopic (LM), electronmicroscopic (TEM, SEM) photos or drawings. Species are described not only by morphological features and distribution areas but also by their occurrence and abundance in different red tide and water bloom types both in Japanese and English. Unfortunately the subscriptions of the figures, important parts of the determinations are written only in Japanese (were not translated into English). A short list of the literature follows every description helping the reader to find more information on the original papers.

The descriptions of the species are accurate and detailed. The figures and the text contain enough information to use them for the determination of the species. The LM micrographs are usually of good quality, the printing is reasonable. Obviously, small organisms such as *Micromonas pusilla* and other species are much more difficult to photograph. The quality of the TEM and SEM pictures is excellent.

It is questionable why several species i.e. *Cyclotella cryptica* Reiman, Lewin et Guillard, *C. meneghiniana* Kützinger, *C. striata* (Kützinger) Grunow, *Thalassiosira guillardii* Hasle, *Th. weissflogii* (Grunow) Fryxell et Hasle, maintaining only species from the Centrales order of Bacillariophyceae are included in a book on red-tide organisms while a number of Cyanophyceae species with high individual number in water blooms are not. I think the authors did not, could not want to publish only the true red-tide, water bloom organisms.



I warmly recommend this brilliantly illustrated, greatly informative book on red-tide organisms, their blooms and toxicity to all algologists, hydrobiologists and ecologists. You can also use it when determining algae! Besides, the book is of good use in basic research and university education.

K. T. KISS

POPOVSKY, J., PFIESTER, L. A.: Dinophyceae (Dinoflagellida). — In: Ettl, G., Gerloff, J., Heynig, H., Mollenhauer, D. (eds): Süßwasserflora von Mitteleuropa, Band 6. — Fischer Verlag, Jena, Stuttgart, 1990. 272 pp.

In certain respect this volume is different from the others of this series. On the one hand it is written in English (all the others are in German), on the other hand the Dinophyceae are a special group of protists. As it is written in the preface: "This volume compilation of organisms which are of interest to phycologists and protozoologists. Since it is very difficult to classify the unicellular organisms as plants or animals in the classical sense, two approaches can be found in the literature to the classification of dinoflagellates — the botanical and zoological.

We have employed the phycological (botanical) approach in our textbook from a practical aspect. However, we view the dinoflagellates as protists which exhibit both plants and animal features. This also holds true for the marine dinoflagellates which are richer in number of genera and species than their freshwater counterparts."

The book starts with a short traditional preface of editors and the determination key of algal classes. At the beginning of the general part we can read in detail about the cell covering, which has a special structure and is the most important characteristic of the determination. Also in the general part the other elements of cell structure (flagella, protoplasm, dinokarion, chloroplasts, vacuoles, pusules, miscellaneous organelles), the cell morphology (monadoid-, rhizopodial-, coccoid habit), the reproduction (asexual, sexual), the resting stages, the life history, occurrence and ecology are described. There is a lot of important information about the sampling-, identification- and culture methods.

The special part is started with the key of two subclasses of Dinophyceae (Adinophycidae, Dinophycidae), which is followed by the short description of subclasses, the keys of orders, the families and the genera. There is a short description of the genera and the key of the species. Finally the authors present the morphological features of each species, showing the important characteristics with which we can determine these algae. Many pictures help the identification. There are also data about the occurrence of the species.

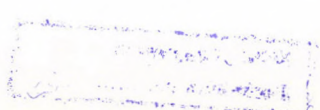
On the basis of the keys, the plate formulas of the epitheca and the hypotheca, the drawings of the cell shape or diagrammatic plate arrangements and the morphological descriptions it is not so difficult to determine of Dinophyceae species.

Recently many interesting papers have been published about electronmicroscopical (EM) investigations of the theca structure of Dinophyceae. If EM micrographs were present the book would be of better use.

It is very useful that this book has been published because it helps to extend our knowledge about the taxonomy and ecology of Dinophyceae, which is needed as recently there are many poorly described species with only few ecological data. "However, we cannot compare the few ecological studies conducted on freshwater dinophyceans to those of other freshwater algae because the present knowledge of their biology is too incomplete. Further, they are often overlooked or misidentified in samples. Thus, it is not often possible to correlate their occurrence with physical and chemical factors in aquatic studies of algal communities" — is written on page 73.

The book is a very useful one, especially for those experts and researchers who have already acquired considerable knowledge in the topic. Besides algologists, we can recommend it without hesitation to hydrobiologists, taxonomists both to zoologists and university teachers.

K. T. KISS





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1. Title, name of the author(s), affiliation, dateline, abstract, keywords
2. Text, acknowledgements
3. References
4. Footnotes
5. Tables
6. Figure captions
7. Illustrations

1. The *affiliation* should be as concise as possible and should include the complete mailing address of the authors. The date of receipt of the manuscript will be supplied by the editors. The abstract should be in English and should not exceed 250 words. It should clearly and simply summarize the most important methods and results. 5–10 significant expressions describing the content are used as keywords. Authors may recommend these keywords.

2. The *text* of the papers should be of high stylistic standard, requiring minor corrections only. The section headings, names of authors cited, latin names and terms should *not* be underlined or in capitals. Quantities should be expressed in SI-units.

3. *References* are accepted only in the Harvard system. Citation in the text must be as: ... (Jakucs 1961)... or Jakucs (1961)...
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The list of references should contain the names and initials of all authors (the abbreviation et al. is not accepted here); for *journal articles* year of publication, the title of the paper, title of the journal abbreviated, volume number, first and last pages.

For *books* or *chapters in books*, the title is followed by the publisher and place of publication. Book title words should be written with majuscules. Titles of papers published only in Hungarian should be translated in parentheses. All items are recommended to be cited both in the text and references.

Examples:

Jakucs, P. 1961: Die Phytozöologischen Verhältnisse der Flaumeichen-Buschwälder Südostmitteleuropas. Akadémiai Kiadó, Budapest.

Fekete, G., Précsényi, I. 1981: Niche structure of a perennial sandy grassland. In: Stefanovits, P., Berczik, Á., Fekete, G., Seidl, M. (eds): Man and the Biosphere Programme. Survey of 10 Years Activity in Hungary. Budapest, 68–102.

Borhidi, A., Muñoz, O., Del Risco, E. 1979a: Clasificación fitocenologica de la vegetación de Cuba. Acta Bot. Hung. 25: 263–301.

Jakucs, P. 1973: „Sikfőkút Projekt”. Egy tölgyes ökoszisztéma környezetbiológiai kutatása a bioszféra program keretein belül (Sikfőkút-Project. Environmental-biological research of an oakwood ecosystem within the framework of the Biosphere program). MTA Biol. Oszt. Köz. 16: 11–25.

4. *Footnotes* should be typed on a separate sheet.

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*

The editors will send information to the first author about the arrival and *acceptance* of the paper. Two *proofs* will be provided, which are requested to be returned within 48 hours of receipt to the editor. Alterations in the text and especially in the illustrations are expensive and should be avoided. Fifty *offprints* are supplied free of charge.

These instructions are valid as from 1983.

